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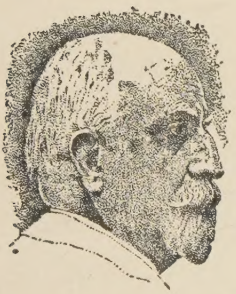

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C. S. I. C.
INSTITUTO «JAIME FERRAN», DE MICROBIOLOGIA

CYTOARJESIS IN POTATO TUBERS *

I. Fundamentals and methods.

BY

ROMAN VICENTE JORDANA

A) REASONS FOR SUGGESTING ACTIVE DEFENCE AGAINST INFECTION IN ORGANISMS

It is generally believed that in host-parasite relationships the whole explanation as to why infection takes place is not available. Twenty years ago MAC CALLUM (1936) made a similar statement and considered that bacteria are not entirely destroyed by systems of defence, which may act as outpost guards: "there is a characteristic flora, including pathogenic forms, waiting, as it were, to break through the second line of guards". Nevertheless, concepts of infection are changing and to-day it is considered that host-resistance may be represented by two systems of defence, both passive and active. In specialized root-infecting fungi forming rhizomorphs, "epiphytic growth over the surface of the host root is necessarily more rapid than growth through the internal tissues, where the rhizomorphs encounter the mechanical resistance of cell walls and the living resistance of 'protoplasts'" (Garrett, 1956b)

From the observation of natural phenomena and the work of seve-

(*) This work was carried out at the Instituto de Edafología y Fisiología Vegetal, Madrid, and at the Botany School, University of Cambridge. The author is indebted to the Dirección General de Relaciones Culturales del Ministerio de Asuntos Exteriores, Madrid, to the Consejo Superior de Investigaciones Científicas, Madrid, and to the Ramsay Memorial Fellowships Trust for the award of Fellowships which permitted him to undertake research in Cambridge.

He would also like to thank Prof. J. M. Albareda and Prof. L. Vilas, of Madrid University, Dr. W. J. Dowson, Dr. J. Rishbeth, Dr. S. D. Garrett and Dr. N. F. Robertson, of the Botany School, Cambridge, for their interest and critical advise.

ral authors it was considered (Vicente Jordana, 1955b, e) that active defences are of the greatest importance in embryonic tissues and are under the influence of meristematic activity. This type of defence apparently operates in a wide range of organisms, both in animals and plants. It is known that some embryonic tissues are almost sterile and female mammals affected by some diseases do not infect the foetus. Antibodies of the mother may be present, although several authors say that antibodies appear after a period of extra-uterine life. Chicken embryos, which may be considered with some exceptions to be free of antibodies and latent viruses, are used to study animal viruses. Callao (1936), from experimental inoculations of mice, found that pregnant females were preserved from infection. Human skin and mucous membranes, also formed by actively regenerating cells, protect the body from infection.

Observations on plant pathology seem also to demostrate that radicular apexes, new shoots and young elements of plants may appear sound while more mature or old tissues can be affected by some specific diseases (Vicente Jordana, 1955e). Bennett (1946) associated the susceptibility of potato tubers to soft-rot with a state of immaturity. Mature tubers were normally sound: "Strong sprouts sometimes grew vigorously directly through the rotted piece, but more frequently sprouts embedded in the rot for many days died off at the tips; however, when removed from the rot a bud developed alongside the dead apex." A state of pregermination was also considered by Focke (1952) to reduce infection of potatoes by *Rhizoctonia solani,* and Boyd and Henderson (1953) found that "as growing potatoes mature their resistance to blight infection increased. This increase of resistance does not appear to be due solely to the increasing mechanical resistance of the skin, for it is found in tubers that have been severely skinned". Bausa (1955) has described an inhibition of the growth of *Aspergillus gracilis* by roots of *Solanum melongena* L. cultivated "in vitro". The phenomenon occurs only when the roots are alive and in young cultures. Finally, there may be mentioned the study of Ferenczy (1956) on antibacterial substances in seeds; when seeds of 400 species and varieties of plants were germinated, 36 species gave positive results. Antimicrobial activity of extracts of chicken embryos, potato buds and other germinated seeds under optimal conditions of temperature and ionic balance have also been reported (Vicente Jordana, 1955e, 1958).

Evidence suggesting that special systems of active defence could take part in arresting infection in a manner consistent with the foregoing examples, was obtained by the writer in 1949. Preliminary experiments with chicken embryos, although useful in some ways, did not fulfil the requirements for a demonstration of this form of defence. Soon after, research with other materials gave encouraging results, as for example experiments with inoculation of germinating tomato and bean seeds, and with wheat, tomato, and bean extracts, but like the experiments with chicken embryos they were found to be incomplete. Experiments with potato tubers were more promising and it was reported that a bacteriostatic effect could be observed when tubers were actively germinating (Vicente Jordana, 1953). Further experiments showed that a state of active germination was apparently necessary to arrest infection (Vicente Jordana, 1954, 1955c) and that the phenomenon did not only concern bacteria, but also fungi (Vicente Jordana, 1955a). For this reason the term "bacteriostatic effect" was considered inappropriate (Vicente Jordana, 1955d, e). Nevertheless this term will be used throughout this work to express the ability for defence through biological activity.

B) THE CONCEPT OF CYTOARJESIS

According to the foregoing reasons it was considered (Vicente Jordana, 1955b, e) that an individual system of cell defence against infection could be developed at some states of cell proliferation.

Since the parasite is also a living organism capable of infection during a state of active proliferation, it is probable that it has an equal chance to exert its own growth energy on the host. Some authors believe that there is insufficient evidence to show that infection is commonly produced by the toxigenic effect of micro-organisms. Except in the case of a few organisms which produce exotoxins, the possibility that endotoxins produce a full syndrome of the disease is far from being accepted. It is known that endotoxins as well as "vivotoxins" may produce partial syndromes, but they are not the initial agents of the disease. Dimond and Waggoner (1953) considered that if lycomarasmin (a vivotoxin) is functional, it probably does not play a primary role in the pathology of *Fusarium* wilt of tomato.

If biological activities play an important role in host-parasite relationships, infection could be considered as a system of two components in which host resistance might be broken down by activity of the parasite. Conversely, development of the parasite could be arrested by the activity of the host and it might be expected that the whole system could reach a state at which both activities were in equilibrium.

Consequently, it was postulated (Vicente Jordana, 1955b) that in the host-parasite system "a state of unstable equilibrium might occur, that component tending to predominate for which conditions for cellular growth were more suitable". From this predominance of one component over the other it was proposed that this antagonistic biological equilibrium phenomenon should be designated "Cytoarjesis" (kytos, cell; arche, to direct, to command) (Vicente Jordana, 1955d). Generically, the term "Cytoarjesis" may be used to describe a particular kind of defence of organisms during processes of cell synthesis.

C) INTEREST OF POTATO TUBERS

Under those suppositions, the experiments reported in this work were planned to ascertain the facts and to try as far as possible to obtain evidence on the role of biological activity in the processes of infection and defence. As stated by Garrett (1956a): "...by careful observation of facts and collation of data, much was learnt about the behaviour of... microbiological phenomena before their cause was understood or even dimly apprehended". Although the evidence obtained may be exclusively concerned with the potato tuber itself, this is thought to be valuable for the purpose in having the following advantages over other organisms (animals and plants):

1. Its great autonomy. Suitable conditions are the only requirement for a potato tuber to develop its germinative processes.

2. Its considerable susceptibility to soft-rot. Although Stapp (1947) established that there are several degrees of genetical resistance to this infection, no variety is known to be wholly resistant. All 21 varieties used by the writer were susceptible to experimental inoculation with several micro-organisms as well as to natural infection. Genetical resistance to

other diseases, such as late blight, dry-rot, wilt, internal cork, nematode root-knot, etc., has been reported.

3. Its inability to produce antibodies. As with other plants, there is no evidence that reactions of the potato tuber to infection include antibody formation.

4. Its ability to stop or to re-start germinative processes under experimental conditions. Germinative activity may be modified by temperature and aeration.

5. The autonomy of buds allows different experiments to be carried out with the same tuber. A tuber can be divided into two or more parts and each acts independently of the other. Thus it is possible to discover the different behaviour and reactions of potato tissues with varying degrees of activity.

The phenomenon, studied in nine Spanish varieties, has also been observed in twelve English varieties. Since experiments started, over 4,000 single observations of decaying and healthy material have been made. The phenomenon seems to be general and, as said above, to be unconnected with varietal differences. The possible role of passive defence is taken into consideration. The formation of wound periderm and the presence of solanin or other antibacterial substances have been considered by the writer to be inadequate entirely to account for defence of the tuber. Recently McKee (1955) has suggested that solanin may play some part in restricting fungal infection. Chlorogenic acid is also associated in modern papers with resistance of potato tubers to infection, mainly by the causal organism of scab.

Possible interference of viruses was taken into acount when some selected tubers used in the experiments gave a percentage of 1 % of virus A and 1 to 2 per cent of virus X for varieties Alava and Sergen, these being free from other viruses (*).

D) CHOICE OF MICRO-ORGANISMS

The organisms used throughout the previous and present work for experimental inoculations mainly belong to the *Erwinia carotovora* group, which from the work of Harding and Morse (1909) and more recently

(*) Data obtained from the Estación para Mejora de la Patata, Vitoria (Spain).

from that of Dowson (1949), Rudd Jones (1948, 1950) and Hellmers and Dowson (1953) are considered to be varieties of the same organism. These varieties are *aroideae, phytophtora* and *atroseptica.* Since these varieties have a lower production of enzymes other than those common to simple bacteria and no mechanism is known of their activity and development on potato tissues, it might be considered that they could give a suitable organism for parasite. According to Jones (1909), *E. carotovora* does not produce enzymes other than those dissolving the middle lamella. Later, Wood (1955) has described two pectic enzymes which can be found in filtrates of *E. aroideae* growing in a synthetic medium; one is a depolymerase and the other a protopectinase. More recently, Echandy, Van Gundy and Walker (1957) have found that these 3 common soft-rot bacteria produced an abundance of depolymerase. No evidence of pectin methyl esterase and no polygalacturonase was obtained. Comparable preparations of the enzymes showed that those of *E. carotovora* and of *E. atroseptica* produced a more rapid maceration of carrot tissue than that of *E. aroideae* at their respective optimum pH levels (pH 6,5 for the former strains and 8.5-8.9 for the latter). According to the authors, there was no indication that pectolitic activity was associated to the property of inciting black leg of potato.

Bacillus polymyxa, which is considered to be one of the most actively saccharolytic and proteolytic bacteria and was described by Dowson (1943) to produce a severe rot under conditions of high temperature and saturated atmosphere, was often used in the investigation. Other bacteria and fungi have also been used: for instance some tests were made with *Preudomonas marginalis, Ps. syringe, Phytophthora infestans* and *Fusarium caeruleum.*

Differences of pH, found betwlen healthy tissues and rotted material may be associated with the physiological condition of each component of the host-parasite system. These differences have also been noted by other authors. Healthy tissues normally have a pH of about 6 and infected ones are alkaline, though showing some variation.

E) MATERIAL AND METHODS

1. *Basis of the methods.*

a) *Purpose.*

The main problem was to find a method by which the activity of both parasite and host would be correlated, in order to establish the interaction between them. After that, if the bacteriostatic effect was really due to biological activity, the partial inactivation of either component of the host-parasite system would permit the exponential representation of the phenomenon and the localization of the active parts of the host defence.

The exponential curve would demonstrate the equilibrium of both activities and would show each of their maxima as opposite.

b) *General development.*

Since no references could be found in the literature of this type of study, methods were based on general techniques of biology and those of microbiology and plant pathology. Special attention was paid to information relating to experiments made with potato tubers.

For each experiment, and before detailed results were obtained, it was necessary to derive experience from pilot tests.

c) *Assessment of the Host-Parasite Activities.*

The problem had two main aspects. It was thought that information could be obtained with one by measuring the development of buds as an index of the activity of the host and with the other by measuring the increase of soft-rot, giving the activity of the parasite.

Since assessments of host activity can easily be obtained by weighing buds, to measure activity of the parasite under several conditions by a standard method was the main task of the experiments concerned in this work.

As often happens in biological techniques, the methods of inoculation, conditions of incubation, etc., used to obtain experimental infections in potatoes, as well as measures of increase of soft-rot, vary greatly with the investigator. A standard method has not yet been accepted for general

use and the results so far obtained are related to the various systems used by different authors or schools of research. Frequently results are obtained empirically or are expressed as percentages. At present, correlation of results from several authors may, in many cases, do no more than permit us to say whether tubers were rotted or not and, occasionally, how severe the infection was.

Attempts to establish a standard method have already been made and interesting data can be collected from the relevant literature. Some of the more accurate methods developed in this work were mainly based upon the work of Rudd Jones (1948) and Boyd (1952).

d) *Methods of Inoculation and Evaluation of Soft-Rot.*

For purposes of *inoculation* three main methods of wounding the tuber have been described in the literature: (a) by stabbing or piercing it with metal or glass instruments, (b) by making superficial cuts or scratches, and (c) by cutting the tuber into several pieces

Inocula consist of bacterial grown in solid or liquid media or of bacterial suspensions in several liquids ranging from sterile water to nutrient broth. Inoculum is introduced at the time of wounding or later by means of a platinum loop, Pasteur pipette or similar instrument. Pieces of agar supporting bacterial growth or infected tissue may be used as inocula and the employment of a hypodermic syringe to deliver the required volume of inoculum has also been described.

As plugs of potato tissue may obstruct the hole of a direct inoculator, different specially-made instruments can be used. Rudd Jones (1948) for some experiments used a special *cork-borer* (diameter 2 mm.) with a shoulder-stop constructed so that a plug of tissue exactly 2 cm. in length could be removed. McKee and Boyd (1952), for inoculating soil into potato tubers, used a glass inoculator with a round end.

A new method, using a modified hypodermic syringe to inoculate *Fusarium caeruleum,* has been described by Boyd (1952). The needle in the needle block was cut off and the hole soldered, while another hole 1 mm. in diameter was bored in the side of the needle block 2 mm. from the end. The tuber could thus be wounded to a uniform depth of 7 mm. and the inoculum calibrated. Pen Ching Cheo (1953), to inoculate sweet potatoes with spores of *Endoconidiophora fimbriata,* used

a straight medicine dropper having a sharp tip 2 mm. in diameter, delivering inocula to a depth of about 8 mm.

The *amount of rot can be assessed* by measuring the diameter and detph of the lesions (Boyd, loc. cit.). Rudd Jones (loc. cit.), after cutting the tubers lengthwise through the point of inoculation, measured the extent of the rot in the storage parenchyma and then its width by a further cut at right angles. The mean of the two readings was taken as measure of the amount of rot produced. In some instances the same author used a method involving weight of rotted tissue. Garrett (1956) measured the extent of infection by *Armillaria mellea* on potato tubers by means of the fresh weight of infected tissue and the radial extent of infection. Smith and Smart (1955), after inoculating slices with a standard number of drops, gave to each slice a numerical rating based on the area decayed, to express the development of soft-rot.

2. *Material and apparatus.*

The following varieties of potato were used to a greater or lesser extent: Palogan, Victor, Sergen, Alava, Frühgold, Gauna Blanca, Turia, Heida, and Roja Riñon; Sharpe's Express, Majestic, King Edward, Chancellor, Doon Star, Eclipse, Arran Pilot, Arran Peak, Catriona, Ulster Prince, Ulster Supreme and Dr. McIntosh.

Tubers were grown at the Instituto de Edafología y Fisiología Vegetal (Madrid), at the Facultad de Veterinaria field (Puerta de Hierro, Madrid), at the Cambridge University Botanic Garden, or at the Rockefeller Field Station (Cambridge), or obtained through the courtesy of the Estación para Mejora de la Patata (Victoria, Spain), Servicio de la Patata de Siembra, Ministerio de Agricultura (Madrid) and the National Institute of Agriculture and Botany (Cambridge).

As experiments in this work were performed "in vitro" conditions, containers of various sizes were used. Petri dishes were employed for slices, glass-stoppered bottles for single tubers and crystallizing dishes, china containers or large plastic boxes for groups of tubers. For humidity tests, tubers were at first placed on glass tripods in closed containers but this system proved unsatisfactory because the presence of free moisture was found to be necessary for bacterial growth. Later tubers were placed or buried in peat, sand or pumice powder, to obtain the

desired conditions, and were usually wrapped in pieces of muslin to separate them from particles of the substratum.

Glass and china apparatus were dry-sterilized or autoclaved and larger containers after thorough washing were swabbed with 95 % alcohol.

3. *Methods.*

The main methods used are described below. Further details of certain experiments will be explained as necessary.

a) *Preparation of the Tubers.*

If tubers had sprouted, it was not found necessary to submit them to any special preparation in order to carry out the experiments. It was advisable to use tubers in which the germinative activity had been stimulated.

Sprouting of dormant tubers was obtained by first placing them at temperatures between 18-20° C in dry conditions. These conditions often protected the resting tubers from an early development of latent infections, particularly evident at higher temperatures and in moist conditions.

After buds had appeared, tubers were transferred to more stimulating temperatures and humidities. A temperature of 22.5° C and a moisture content of 20-50 % by weight in pumice powder were satisfactory. It was desirable, however, not to cover the tubers with a deep layer of pumice when the moisture content was over 30 %. The tubers could also be stood (but not immersed) in a small volume of water or laid on filter paper over-lying moist sand or peat.

Temperatures over 28° C. or below 5-6° C. were avoided, since they led to temporary or sometimes also permanente damage or inactivation of tubers.

If tubers were placed on trays or open containers it was advisable to avoid their exposure to light by covering them with black cloth or paper.

b) *Cleaning and Aseptization of Tubers.*

As a preliminary, tubers were cleaned by gentle brushing with a hard-bristle brush in running tap-water. The use of soaps. detergents or

chemicals was deliberately avoided to ensure that no disinfecting substances of any type impregnated the tubers and affected the results. In consequence, the importance of meticulous cleaning is obvious. Notwithstanding, to obtain material as free from contamination as possible, the tubers, after drying, were treated lightly with alcohol. Subsequent flaming of the tubers was optional. Experiments showed that very brief exposure to methyl or ethyl alcohol had no detrimental effect on germination, but, when done, had to be completed in a few seconds, otherwise inactivation of the tubers occurred.

This system proved good enough to stop secondary infection at the first stages of experimental infection and the general condition of the tubers seemed not to be affected. Pure cultures of the test organism were obtained several times from the lesions five days after inoculation and on many other occasions examination of stained smears showed the characteristic Gram-negative rods. After 10 days incubation at 22.5°-C. in aerobic conditions, the appearance and odour of the rotten tissues also indicated the absence of contaminations. On three occasions, confirmation of this was obtained by isolating the inoculated organism in pure culture.

Nevertheless secondary infections almost always appeared, especially in inactive tubers. This occurred even more rapidly when anaerobic conditions or high temperatures of incubation were involved.

Since tubers must be treated in such a way that neither they nor the bacteria are damaged, the system of partial sterilization used was far from being perfect. Special observations were made on contamination during one of the experiments, after 13 days, when tubers of Sharpe's Express variety were covered by pumice powder having moisture contents of 20, 30, or 40 %.

As it was often difficult to observe contamination on active tubers, the presence of micro-organisms was investigated on the muslin with which the 30 uninoculated tubers had been enveloped. To a greater or lesser extent, depending mainly on the activity of the tubers, all the pieces of muslin were contaminated by a pink pigmented bacterium and a green mould. Other organisms were a white fungus and a yellow pigmented bacterium which appeared in some instances, and gas was produced in three of the decaying tubers. No identifications were made. It was apparent that the pink pigmented bacterium attacked cellulose, for

the muslin was holed or was easily torn at the point at which the pink pigment was observed. Contaminations and initial soft-rot were general in inactive tubers.

When the bacteriostatic effect under investigation was to be observed at its optimal development, it was advisable merely to wash the tubers with water. For this unsterilized tap water was used, as it also was, in many instances, for partially immersing the tubers during incubation.

c) *Inocula.*

Special attention for comparative results was paid to the strain of *Erwinia carotovora,* var. *aroideae* (66A), of the Collection of Plant Pathogens, Botany School, University of Cambridge. Other bacteria and fungi from the same Collection were: *Erwinia phytophtora* (strain, no. 138), *Erwinia carotovora,* var. *atroseptica* (strains no. 275 and 409), *Pseudomonas marginalis* (strain no. 247), *Pseudomonas syringae* (strain no. 367), *Bacillus polymyxa* (strains no. 90 and 164), *Phytophthora infestans and Fusarium caeruleum.* Two issolates (*E. carotovora*-like) from rotted potatoes were also used.

Standard inocula were prepared for *E. carotovora,* var. *aroideae,* using heart infusion agar slopes of the following composition:

Horse or Ox heart.....	$^1/_2$ lb.
Peptone.....	10 g.
Common salt.....	5 g.
Yeast extract.....	0.5 g.
Tap water.....	1,000 ml.

The bacterial growth developing after 24-48 hrs. was removed and suspended in 1 ml. of saline isotonic solution, rendered homogeneous by shaking for 5 minutes and left to settle for another 5 minutes. The suspension was then transferred to a sterile tube.

d) *Methods of Inoculation.*

Two methods of inoculation were used during the experiments. One was a general method aiming to produce a large contamination of the potato tissues by the test organism and to obtain, if possible, a heavy

infection. The other was a standard method for obtaining comparative and more accurate results.

The technique for *method* (1) has been previously described (Vicente Jordana, 1954) and involved the production of one or several deep wounds by piercing right through the tubers with a long sterile steel punch (1.5 mm. diameter). Inoculum was inserted into the wounds by a sterile Pasteur pipette. Very often large internal wounds were produced by twisting the punch and sometimes the tubers were almost broken into two parts, held together only by the skin and some parenchyma tissue.

Results were obtained after the required time by direct observation of the tubers, and rotten and healthy material was selected in order to obtain the percentage incidence of infection and for photographic purposes.

For *method* (2) smaller wounds in the tubers were made by a small steel borer (2.5 mm. diameter and 5 mm. long) mounted on a steel handle. The borer was flamed and immersed in distilled sterile water before being driven into the potato tissue. Once the piece of tissue had been extracted, one or two droplets of inoculum were placed inside the hole by means of a Pasteur pipette, calculated to deliver 30-40 drops per ml.

After inoculation the tubers were either left unwrapped or were wrapped in muslin or other types of cloth: they were then placed in the substratum or directly in contact with water. After wrapping, if necessary, tubers were then incubated at the desired temperature. Envelopment of tubers in cloth was advisable when pumice powder, sand or peat was used and was essential under these conditions when the weight of rotten material or of the buds was required. Cellophane was avoided (especially with peeled tubers or slices) because it caused a thin layer of moisture round the tuber and produced a putrid mass hindering measurements.

Soil and pumice powder were the best substrata for most purposes. Sand was less suitable for measuring the moisture content but had some advantages for distinguishing between active and inactive tissues in a tuber, the inactive tissues being rotten. Sterile peat often became contaminated to a great extent.

e) *Methods of Assessment.*

The activity of both parasite and host were measured by the extent of rotted material. This extent was determined by the depth and the

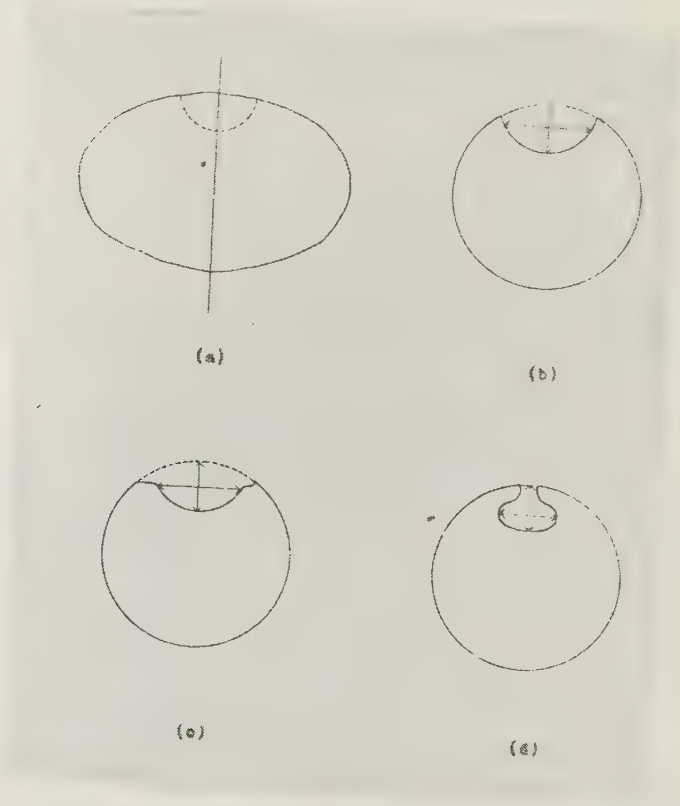


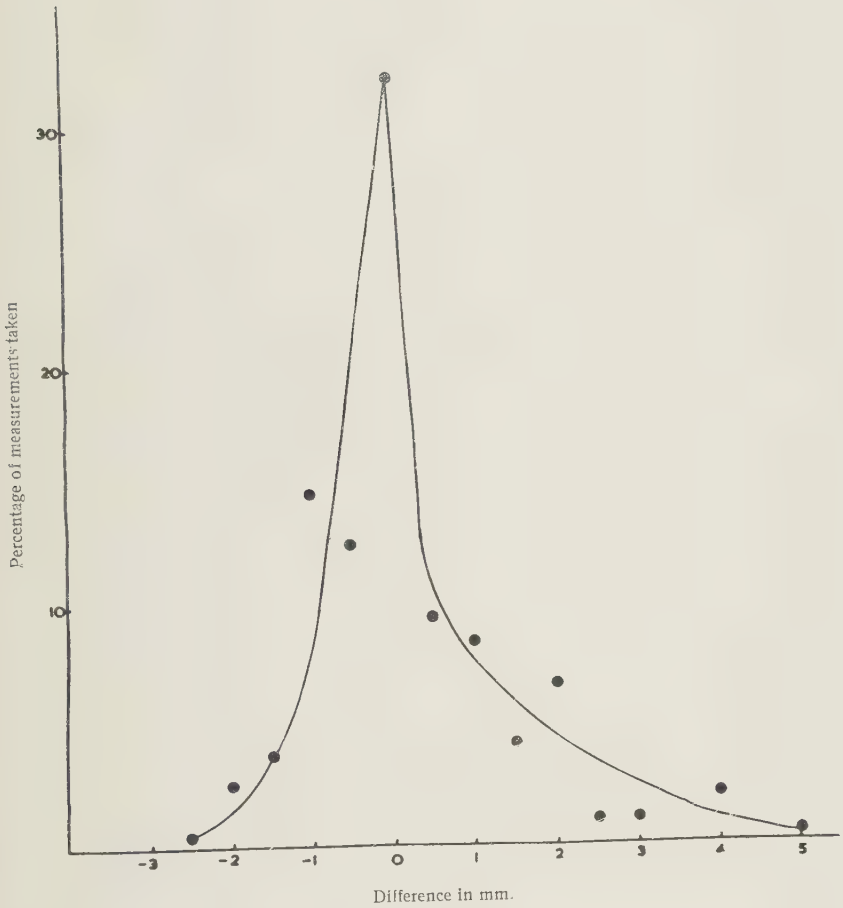
Fig. 1.

external and internal width (in mm.) of the lesions, and also by the fresh and dry weight (normally both) of the decaying tissue.

The external diameter of the lesion was taken by dividers or by the sharp edges of forceps and measured on a calibrated rule. The tuber was then cut perpendicular to its main axis, dividing the lesion into two equal parts, as seen in fig. no. 1(a). Width was measured at about

the middle part of the internal lesion if it was regular in shape [fig. 1(b)] or from the widest clean diameter of the cavity if it was irregular or constricted in places [fig. 1(c) and (d)].

After a tuber had been cut open it was difficult if not impossible

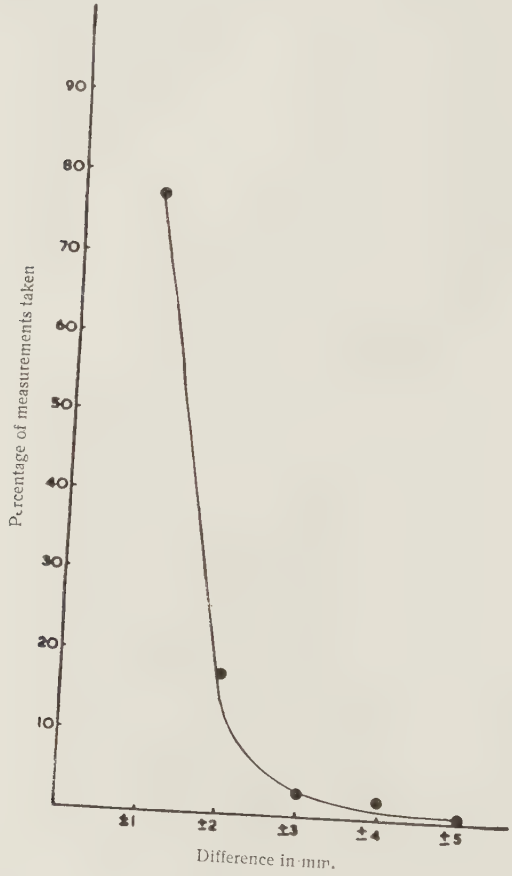


GRAPH 1.

for certain purposes to continue the experiment. Therefore in order to maintain observations on a single tuber, measures were limited to the external extent of the lesion and to its depth. In this case depth was measured by inserting a punch through the soft tissue. This measure is

less accurate than that obtained after cutting: the average difference in depth recorded by the two methods in 180 tubers was 0.7 mm. Those results are shown graphically in graphs no. 1 and no. 2.

When measurements were completed, the whole soft-rotted tissue



GRAPH 2.

was carefully collected in a watch glass and weighed. These operations were carried out as quickly as possible mainly when rot was advanced since the moisture content soon falls by evaporation. For tubers where more than about 25 g. of rotten tissue was obtained an ordinary balance was used. With less than 25 g. of decayed material, weighings were made

on a precision balance. Results were also expressed as dry weight after the material had been heated to 100° C. until the weight was constant.

The quantity of water in the potato tissues or in the decaying matter is derived from the quotient obtained by dividing dry weight by fresh weight. This quotient, multiplied by 100 and subtracted from 100, gives the percentage of water content.

f) *Experimental Inactivation of Tubers.*

As it has been supposed that the phenomenon of tuber defence is due mainly to biological activity, inactivation of tubers was attempted in order to cause a decrease in germination without killing the tissues. Efforts were made to do this in such a way that germinative cells would later be able to recover their activity if desired. It was also considered to be of interest to separate active and inactive parts of the same tuber, to observe any differences in their behaviour.

Initially, it was intended to inactivate tubers by means of radiation (X-rays, infra-red, and ultra-violet) and chemical inhibitors of growth, but it was thought later that these would lead to complete inactivation or interfere with bacterial activity. Consequently, with the single exception of using alcohol, no inhibitors were employed. Six methods of more natural inactivation were selected as follows: (*).

(1) Removing buds and their germinative points inside the tuber by means of a knife or scalpel.
(2) By cutting the tuber into slices either bearing or not bearing buds.
(3) By peeling the tuber, leaving active points if required.
(4) By altering the temperature of germination.
(5) By covering the tuber with pumice powder having a moisture content of at least 30-35 %.
(6) By immersing the tuber in water.

(*) Recent experiments have shown that the effect of light on tuber inactivation is not so great as had previously been supposed (Vicente Jordana, 1955a). It is now believed that temperature could have considerably influenced the results of the previous experiments.

Summer temperatures in Madrid often exceed 30° C, and since the exposure of potato tubers to light then took place at room temperature it is highly possible that the inactivation of the tubers resulted from this factor. At the time of the publication referred to above (Vicente Jordana, 1955a) the exact effect of temperature on tuber inactivation was not so well known (see Vicente Jordana, 1955c).

g) *Measuring Germinative Activity of Tubers.*

The fresh weight of developed buds has been used as a measure of the activity of tubers in previons work (Vicente Jordana, 1955c).

Buds of selected tubers were removed gently with the fingers and, if required, carefully with a scalpel, avoiding damage to the germinative points of the tubers. At the end of an experiment the new sprouts were taken off at their base by the scalpel, placed on a watch glass and weighed. To obtain the dry weight, the material was squashed against the watch glass and desiccated till constant weight was reached at 100° C.

The weight of buds seems to be the most useful measure for obtaining comparable results and to give the most reliable estimate of germinative activity: however the number of grams does not exactly correspond with the extent of the bacteriostatic effect.

It is considered that *a tuber is mature* for the bacteriostatic effect when infection is arrested for at least ten days in the whole tuber. Thus, the terms "maturity" or "mature tissues" used in this work are related to the effect of arresting infection under the influence of developing buds. "Active tuber" means that buds are active and "inactive tuber" is used when tubers have, under natural or experimental conditions, stopped the normal develoment of buds.

Thus, where a number of tubers have been in the same environmental conditions from the beginning of an experiment, the weight of the buds would express for each tuber the state of maturity or inactivation of the germinative tissues but would not express in many instances the pontentiality for defence. At an early stage of development some tubers have immature tissues susceptible to rot whilst mature ones resist infection. Under these conditions buds may develop to an extent unrelated to the rotten material of immature tissues if results are to be compared with those of fully mature tubers. On the other hand, increase in the size and weight of sprouts is linked to external factors, such as temperature, humidity, air conditions, time, former time of storage, etc., which will account for differences between individual tubers. Also, it was found that damage, previous temperatures at which tubers were kept, former dryness, potential infections, etc., may affect sprouting.

Although in many experiments measures of sprouting activity involving removal of buds and those of the increase of soft-rot were ca-

rried out on the same tuber, it is advisable for observing the bacteriostatic effect to inoculate tubers on which buds are developing. Sometimes tubers which were expected to develop buds did not sprout again, and soft-rot started if conditions were suitable. Sometimes this was due to an inadequate treatment with alcohol. It is also possible that infection to some extent prevents sprounting in some partially inactive tubers, especially in tests involving high humidities and poor aeration. Development of fungi has been observed in stumps of supressed buds and occasionally on living buds. At other times, after removal of well developed buds, stumps were covered by a thick layer of periderm and this may have hindered development of new sprouts. Bennet (1946) considered that some varieties (Arran Pilot, for example) may not sprout after removal of buds, but in the present experiments no attention was paid to this aspect of varietal differences. Activity was in fact measured by the weight of new buds produced after removal of existing ones. It is clear that other factors besides variety affect sprouting.

SUMMARY

As previously reported, some biological phenomena suggest that active defence of organisms against infection is of the greatest importance in embryonic tissues and in those under the influence of meristematic activity. Since the parasite is a living organism capable of infection during a state of active proliferation it is probably able to exert its own energy of growth on the host. In this case, if biological activities play an important role in host-parasite relationships, infection could be considered as a system of two components in wich host-resistance may be broken down by activity of the parasite. Conversely development of the parasite could be arrested by activity of the host, and it might be expected that the whole system could reach a state in which both activities were in equilibrium.

Under this supposition the experiments concerned with this work are based upon the study of the development of soft-rot and other associated infections in potato tubers in various states of germinative activity. The methods were developed in order to correlate the activity of both parasite and host and to try to establish the interaction between

them. Methods of assessing host activity by measuring the weight of buds developed and activity of parasite by weight of soft-rotted tissues, as well as a standard method of inoculation, and other methods of preparation, inoculation and inactivation of the tubers, are described. Since 1951, over 4.000 single observations and experiments of decaying and healthy material (involving 21 varieties of potato) have been made.

Since the equilibrium in the host-parasite system may be broken down, that component tending to predominate for which conditions for cellular growth were more suitable, it was proposed that this antagonistic biological equilibrium phenomenon should be designated "Cytoarjesis" (kytos, cell; arche, to direct, to command). According to the fact that an individual system of cell defence seems to be developed at some states of cell proliferation, generically, the term "Cytoarjesis" may be used to describe a particular kind of defence of organisms during processes of cell synthesis.

RESUMEN

De acuerdo con publicaciones anteriores, algunos fenómenos biológicos parecen indicar que los tejidos embrionarios y aquellos sometidos a la influencia de la actividad meristemática, presentan una mayor capacidad para la defensa activa de los organismos vivos frente a la infección. Teniendo en cuenta que los parásitos son también organismos vivos que desarrollan su actividad patógena durante estados de proliferación activa, es muy probable que su propia energía de crecimiento sea uno de los factores más importantes del proceso de infección. En este caso, habría que pensar que las relaciones huésped-parásito están influídas mutuamente por la propia actividad biológica de cada uno de los elementos que intervienen y que, por tanto, el proceso de infección prodría considerarse como la resultante de un sistema de dos componentes, donde la resistencia del huésped queda rota por la actividad del parásito. De la misma forma, si el desarrollo del parásito quedase paralizado por la actividad del huésped se podría esperar que, en conjunto, el efecto de ambas actividades del sistema alcance un estado de equilibrio.

Partiendo de esta suposición, las experiencias cuyos métodos se describen en este trabajo, están basadas en el estudio del desarrollo de la

putrefacción blanda de la patata, y otras infecciones asociadas, durante ciertos estados variables de la actividad germinativa de este tubérculo y son un complemento de los estudios esbozados en trabajos anteriores. Los métodos se han desarrollado en orden a comparar las actividades biológicas de crecimiento del parásito y del huésped, y tratan de establecer la interación entre ambos. La actividad del huésped se midió pesando los brotes desarrollados, la del parásito por el peso del tejido putrefacto. Se describen ambos métodos, así como el método "standard" de inoculación utilizado y otros relativos a la preparación, inoculación e inactivación de los tubérculos. Como ya se dijo en otra publicación, y se repite aquí, el tubérculo de patata presenta ciertas características especiales frente a otros organismos (animales y vegetales) para el estudio experimental del supuesto equilibrio biológico del sistema huésped-parásito. Desde 1951, se ha utilizado en experiencias y observaciones individuales de material sano o putrefacto un total superior a los 4.000 tubérculos, incluyendo 21 variedades de patata.

Considerando que el citado estado de equilibrio es inestable, puesto que el predominio final será de aquel elemento que se encuentre en mejores condiciones para su desarrollo celular, se propuso el nombre de "Citoarjesis" (kytos: célula; arjé: dirigir, mandar) para la denominación del fenómeno, caso de que tenga confirmación general. Dado que el fenómeno parece ser debido al desarrollo de un sistema individual de defensa celular durante la fase de crecimiento, el término "Citoarjesis" podría usarse, genéricamente, para designar un estado particular de defensa de los organismos durante los procesos de síntesis celular.

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CYTOARJESIS IN POTATO TUBERS

II. Susceptibility of living potato tissues to soft-rot: Effect of inoculum potential *

BY
ROMÁN VICENTE JORDANA

A) CONDITIONS FOR EXPERIMENTAL INFECTION

As said before (Vicente Jordana, 1958), infection may be considered as an ideal system of two components in which host resistance may be broken down by activity of the parasite. "It is now generally recognized that the symptoms of a parasitic disease arise largely from an interchange of chemical substances between the invading parasite and its host" (Allen, 1953). From another point of view, infection could be considered as the consequence of an organism occupying its ecological niche. In general a parasite is a more simple form of life and it may be expected that nutritional factors become more readily available from the host, than from other sources. Also the physiological condition of the host may be important; for example, for some obligate parasites temperature of the host is an essential factor, and some others require fresh material from the host to develop in artificial media. Owing to its simplicity, the development of a parasite in living matter is limited by the physiological and immunological states of the host which are more complex and complete. Organisms are often exposed to their potential parasites, but infections are less frequent than expected from the virulence of the infectious units.

For infection to take place it seems necessary in the *first instance for the host to be weakened,* leading to impairment of its defences and

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consequently to its susceptibility to the parasite. *A second condition is the potency of the parasite,* as determined by the number and strength of its infective units: this affects its ability to overcome the various defensive mechanisms of the host.

As quoted by Garrett (1956a), occurrence of disease must fulfil three conditions, (i) presence of a susceptible host (plant), (ii) presence of and infective parasite, (iii) occurrence of suitable environmental conditions for penetration and infection of the host by the parasite.

These three conditions were taken into consideration in studying the effectiveness of inoculum in breaking down the resistance of potato tissues to soft-rot.

B) SUSCEPTIBILITY OF THE HOST. CHOICE OF METHOD INVOLVING PEELED TUBERS

Since weakness of the host may be one condition for its susceptibility to infection, potato tubers in their natural state were not useful for studying the increase of soft-rot under experimental conditions. In particular, actively germinating tubers proved very resistant to high concentrations of inoculum. On the other hand, the activity of apparently dormant or resting tubers cannot be estimated by sight and it often happens that tubers sprout during an experiment or soon after. Under these conditions the requisite state of weakness in the host is lost. At the same time, to measure the susceptibility of mature potato tissues to soft-rot without the influence of germinative activity was of great importance, as several systems of what might be called passive defence have been described for potato tubers. However, for some time this was thought difficult as the method would require a special system of inactivation for which the following conditions were considered fundamental:

(1) That all determinations should be done under similar conditions.
(2) That parenchyma tissues disconnected from germinative points should remain if possible alive and to prevent changes in the state of this tissue inoculations should be made as soon as inactivation takes place.
(3) That such tissues would show their susceptibility or resistance in a short period of time.

Two methods of inactivation were initially projected: *a)* Mechanical suppression of buds by cutting off and extracting their germinative points by a scalpel or by burning the eyes with a red-hot punch; and *b)* Physical or chemical inactivation of buds by means of radiation or inhibitors.

The first method soon proved inadequate: It was observed that suppression of visible buds often failed to cause inactivation since new sprouts after some time developed from hidden or unobserved eyes. Sprouting also occurred when burning had been insufficient. This difficulty could be overcome in some instances, and when working with a small number of tubers, since periodical inspection could permit the total inactivation of all buds. It was however fuond quite imposible to inspect large numbers of tubers when they were kept in almost sterile conditions or covered by a substratum.

If some tubers were to sprout after an early inoculation, this would result in the failure of the first condition. The same would apply with the second condition if tubers were left for several days before inoculation, since it is most unlikely that all tubers became inactivated at the same time. Both conditions would fail if tubers were not inactivated on the same day and inoculation took place later.

The second method was found to be impracticable. As buds in mature tubers may be located all over the tubers, the use of radiation or inhibitors would make it possible [as stated in another report (Vicente Jordana, loc. cit.)] to cause death or inactivation of surrounding cells or interfere with the activity of the microorganisms. To concentrate the rays on a single point and to estimate the intensity required without knowing the extent of the active tissues was considered to need special research. Furthermore the results of any such technique, as affecting activity or death of cells, would be uncertain.

The question of time was also involved. Allowing that only the buds or their germinative points would be inactivated, uncertainty as to whether new sprouts would emerge would necessitate leaving the tubers for some days or weeks before experimenting with them. The parenchyma in some of the tubers might lose its activity at the moment of inoculation, while in others it might still be active.

A third method using peeled tubers, although not perfect, seems to fulfil all the three conditions and to avoid some of the disadvantages already mentioned. It is based on the method used by plant pathologists to identify and check the pathogenicity of soft-rot bacteria. Potato slices kept in an adequate state of humidity are rotted by *Erwinia carotovora* and similar organisms in a short time. Rot is apparent after incubating 24-48 hours at temperatures over 20° C. even though the tubers were in active germination at the time of cutting and preparation.

The tubers were peeled by a superficial cut in the skin, and buds with their active points attached were removed at the same time. For some experiments buds were left, so that their influence on the defence of peeled tubers might be observed (see plate no. 3, Vicente Jordana, 1955b). After inoculation, the peeled tubers or slices were stood on or covered by the substratum or placed directly in contact with water.

This method has the disadvantage that the superficial rot is very irregular in extent and spreads more quickly than the rot inside the tuber. In many instances the weights and superficial diameters of rotted tissue may not correspond when two similar peeled tubers are placed together in the same container. The distribution of this rot mainly depends on the extent to which moisture is present on the tuber surface. To obtain comparable estimates of the activity of a pure inoculum, depth of the lesions may be taken as the most reliable measure.

C) EFFECT OF INOCULUM POTENTIAL

1. *Reasons for taking these measurements.*

Measurements of the increase of soft-rot following different inoculum dosages were carried out for two reasons: *a)* Knowledge of the number of cells of the parasite and of their activity is necessary for experiments on host-parasite relationships in which the active defence of the host is to be studied; *b)* the amount of inoculum and the cultural conditions have varied throughout the wide extent of the experiments. Although in every experiment the pathogenicity of the inoculum was checked, it was considered to be of interest to estimate the activity of the bacteria and

to make sure, as far as possible, that variations in the conditions of inoculation were not a decisive factor for the production of the bacteriostatic effect.

2. *Some considerations about inoculum potential.*

For many years pathologists in both main fields of biology have been concerned with the influence of the number and qualities of infectious units in the development of infection. Bacteriologists, in research where lysis or inhibition of bacterial growth are involved, also realize the importance of inoculum for the accuracy of their results. Besides the concept of the Minimal Lethal Dose used by animal pathologists, there has arisen in plant pahology the concept of inoculum potential to express the infective potentiality of a parasite. Isolation of phages and measurement of antibiotics activity often depend on the inocula used.

According to Garrett (1956a, b) inoculum potential may be defined as the energy of growth of a parasite available for infection of a host at the surface of a host organ to be infected. Therefore, inoculum potential is related to the number of infecting units for a given area of host surface and to the nutritional status of such units. This concept, which must be taken into account in preparing any inoculum to be used for an experimental study of infection, attaches particular importance to the infective potentiality of a single unit.

In the hypothesis of independent action (Meynell and Stocker, 1957) it is postulated that the mean probability per inoculated bacterium of multiplying to causing (or help to cause) a fatal infection is independent of the number of bacteria inoculated. Therefore, there will always be a chance that bacteria initiating fatal infections may be the progeny of only one of the inoculated bacteria (Meynell, 1957). Thus the number of infecting units in a given minimum inoculum should be related to the virulence or potentiality of the single units and infection should develop under the synergicistic action of such units and their progeny. The possibility that there is an equilibrium of cell synthesis between two antagonistic organisms in respect to the development of infection (Vicente Jordana, 1955a) leads to the supposition that an inoculum may under some circumstances be resisted by the host cells. The surviving parasite cells in a strong host and cells of a weak inoculum in a more

susceptible host muy undergo a period of apparent dormancy, but really one of incubation, before starting a progressive increase if infection. This state of dormancy should be connected with the time required for the inoculum to acquire effectiveness, as determined by the energy of individual units and the number by which these units become infective.

Since the inoculum potential is related to the energy of growth of the parasite and since there may be an equilibrium of factors affecting growth in the host-parasite system at the first stages of infection, age of culture and conditions which increase the energy of the single units may affect the potency of inoculum and therefore fewer cells may be required to achieve infection. Adam and Powell (1956), found that the infectivity of *Xanthomonas pruni* increases with age of culture up to 48 hours and later decreases. However, cell counts from plate cultures gave the greatest number of active cells at 72 hours.

3. *Experimental soft-rot in peeled potato tubers with varied inocula.*

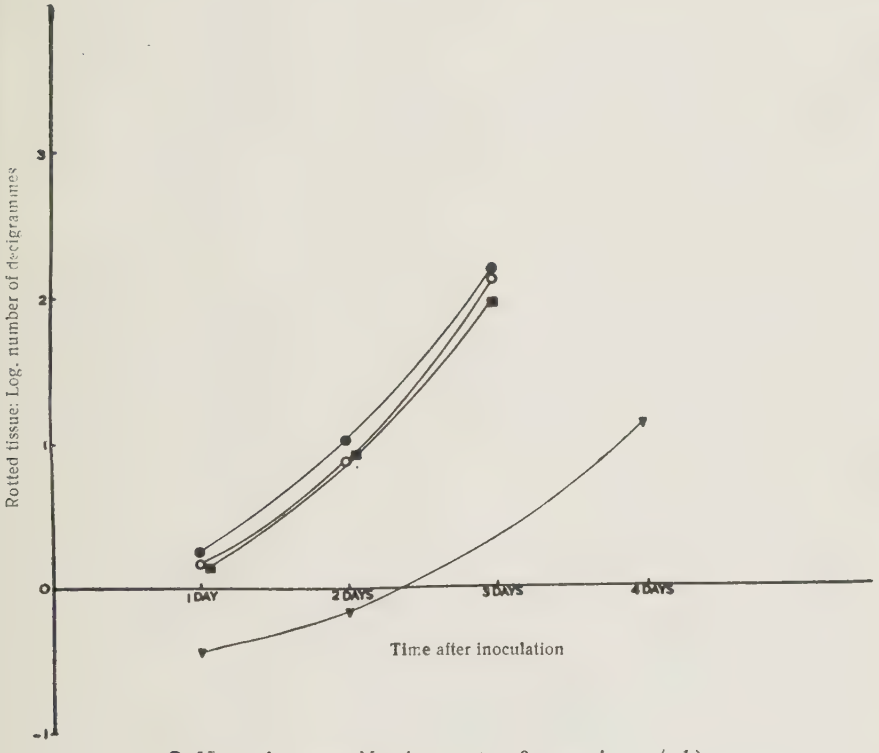
a) *Differences in the number of cells.*

(1) *Inocula.*—Two overnight cultures (15 hrs) of *E. carotovora,* var. *aroideae,* on nutrient (heart infusion + yeast) agar slopes, incubated at 25° C. were suspended in 1 ml. saline solution. After shaking for 5 minutes, both suspensions were mixed and rendered homogeneous. Four inocula were prepared: (i) Undiluted suspension, (ii) 0.5 dilution, (iii) 0.1 dilution, (iv) 0.01 dilution. Dilutions were made in saline solution.

The number of cells per ml. was estimated by the plate count method, using saline for dilutions. 0.1 ml. of each dilution was deposited on nutrient agar-bouillon plates and a count of colonies made after 24 hrs. One droplet of each inoculum was suspended in 10 ml. of saline and further dilutions were made to obtain the exact strength of each inoculum. For the undiluted suspension the mean count of 6 plates was 2.05×10^9 cells per ml. Other values obtained were 2.45×10^6 cells per drop of inoculum at 0.1 dilution and 2.44×10^5 per drop at 0.01 dilution.

(2) *Conditions of the experiment.*—13 peeled tubers of Sharpe's Express variety were used for each of the three more concentrated inocula and 11 for that at the 0.01 dilution. After inoculations by stan-

dard method (2) (Vicente Jordana, 1958), tubers were wrapped in muslin and placed over pumice powder with a moisture content of about 40 % water. Muslin was marked with several coloured dyes to differentiate inocula. Units inoculated respectively with the normal suspension, 0.5 and 0.1 dilutions for measurement after 24 hrs. and 48 hrs. were placed in several containers together with those inoculated with the 0.01 dilution for measurement after 4 days. Each container held representative samples of tubers with the different inocula. This was done in order to obtain similar conditions at early stages of infection, at which pilot



● Normal suspensión (2.05 × 10^9 organisms /ml.).
○ Dilution 0.5.
■ Dilution 0.1.
▼ Dilution 0.01.

GRAPH 1.

Susceptibility of potato tissue to soft-rot.
Effect of inoculum potential on peeled tubers of the variety Sharpe's Express.
Inoculum: *E. carotovora,* var. *aroideae.* Temperature: 22.5° C.

tests showed that the main differences would be found. Pilot tests also showed that at high dilutions inocula showed a preliminary state of dormancy. For this reason the amount of soft-rot comparable to that obtained with heavier inocula was not expected to be produced until 2 or 3 days later.

Tubers for measurement after 3 days and those inoculated with the 0.01 dilution for measurement after 24-28 hrs. were placed in different containers. After measurements some of the tubers were left for 20 days.

(3) *Results.*—Mean from 5 tubers were obtained after 24 hrs. and 48 hrs. for the normal suspension, 0.5 and 0.1 dilutions, and after 4 days for the 0.01 dilution. Results after 3 days and those from the 0.01 dilution after 24-48 hrs. represent means of three tubers. Results are expressed in graph no. 1 and complementary data are collected on table no. 1.

TABLE 1.

Inoculum potential. Complementary data on the increase of soft-rot in peeled potato tubers inoculated with *Erwinia carotovora*, var. *aroideae*. The tubers were enveloped in muslin and placed over pumice powder with about 40 % water content by weight. Average measurements from 5 tubers.

INOCULUM	SIZE OF LESIONS (mm.)						ROTTEN MATERIAL (content of water) %
	E.W. (1) — Hours		I. W. — Hours		D. — Hours		
	24	48	24	48	24	48	48 hours
Suspensión.....	7.0	17.5	6.6	11.7	8.5	12.9	73.7
Dilution 0.5....	8.7	18.2	6.0	10.8	7.1	11.7	72.2
Dilution 0.1....	5.5	15.5	6.6	10.5	7.3	10.9	72.0
Dilution 0.01...	4.0	11.5 (4 days.)	4.0	12.0 (4 days.)	6.0	11.6 (4 days.)	70.7

E. W.: External width. — I. W.: Internal width. — D.: Depth.
(1) Average from ten measurements.

These results show:

(i) That all inocula used were effective for soft-rot production.
(ii) That no great differences were found between inocula till the 0.01 dilution was reached.

(iii) That bacteria were still very active at this dilution or the inoculum (0.01) and soft-rot was comparable (taking into account the time factor) with that produced by heavier inocula.

Tubers with any of the inocula were rotted to such an extent after 20 days that no real differences were found in relation to the inoculum used. Pilot tests with several dilutions up to 0.01 had previously given high percentages of soft-rot for all inocula after 20-30 days.

b) *Nutritional state.*

No special measurements were made on the production of soft-rot with differing states of nutrition or ages of the inoculum culture, but pilot tests showed that both factors should be taken into consideration.

Pilot experiments were carried out with varieties Palogan, Victor, Sergen, Sharpe's Express, Arran Pilot and Majestic (peeled tubers) inoculated with. *E. carotovora,* var. *aroideae,* and other organisms grown on several media. It was found that 24-48 hrs. cultures in nutrient media, after several transfers, seemed a little more effective in the firse stages of infection than inocula from synthetic media. The presence of glucose seemed to give an increase in bacterial activity. *E. carotovora,* var. *aroideae,* grown on Clarke's medium (1952) gave more failures than other inocula, especially when cultures were old.

c) *Age of the cultures.*

Pilot tests were also made in order to measure the activity of inocula in relation to age of culture. Tests of bacterial growth were made by transferring one drop or loop of the cultures to fresh media. Best conditions of soft-rot development were obtained when bacterial growth was stimulated by about one week of successive transfers. No great differences were found between cultures from 24 hrs. to 5 days old. Inocula were effective after 15 days and activity was found in many cases after cultures had been kept for 2 months at room temperature. Old cultures on synthetic media gave more failures. As often happens less active inocula exhibited a state of dormancy before soft-rot really developed.

Normally, failure or reduced activity of an inoculum was linked with failure or slow development of bacteria when transferred to fresh media.

Once soft-rot has started, it develops to a degree equivalent to that occurring with more active inocula.

d) *Conclusions.*

From the experiment recorded above and from observations made during the experiments it may be concluded:

(1) That when peeled potato tubers are inoculated with cultures of *E. carotovora,* var. *aroideae,* at several cell concentrations, nutritional states and ages of cultures, differences in the rate of increase of soft-rot may be found at early stages of infection (3-5 days).

(2) That inocula grown under less suitable conditions may become effective after a short period of apparent dormancy, unless very inactive.

(3) Nearly all the inocula tested were effective for soft-rot production in potato tissues. Frequent checks of pathogenity prove this assertion.

(4) These findings conclusively show that the bacteriostatic effect should not be attributed to failure of inocula.

SUMMARY

If infection may be considered as an ideal system of two components in which host-resistance may be broken down by activity of parasite, it might also be considered from another point of view as if infection were the consequence of an organism occupying its ecological habitat. Owing to its simplicity the development of a parasite in living matter is limited by the physiological and immunological states of the host which are more complex and complete. Consequently, for infection to take place it seems necessary in the first instance for the host to be weakened, leading to impairment of its defences and to its susceptibility to the parasite. A second condition is the potency of the parasite, as determined by the number and strength of its infective units.

According to the fact that actively germinating tubers proved very resistant to high concentrations of inoculum, a method involving the inoculation of peeled tubers was selected to study the susceptibility of potato tissues to soft-rot using varied inocula. Special attention was paid to the differences in the number of cells of the inocula, and pilot

experiments were carried out on the nutritional state and age of the bacterial cultures used for the purpose.

Results showed conclusively that when peeled potato tubers are inoculated with cultures of *E. carotovora,* var. *aroideae,* differences in the rate of increase of soft-rot with varied inocula may be found at early stages of infection. Inocula grown under less suitable conditions may become effective after a short period of dormancy and/or incubation, unless very inactive. These findings show that the bacteriostatic effect should not be attributed to failure of inocula.

RESUMEN

Si, como se dijo anteriormente (Vicente Jordana, 1958), el proceso de infección podría considerarse como un sistema de dos componentes donde la actividad del parásito puede modificar la resistencia del huésped, es posible que también se pudiese decir que la infección no es sino la consecuencia de un organismo laborando en su lugar ecológico. Sin embargo, debido a su propia simplicidad, el desarrollo de un parásito en materia viva ha de estar limitado por los estados fisiológicos e inmunológicos del huésped cuyo organismo es más completo y complejo. En consecuencia, para que la infeción se produzca, parece necesario en primer lugar que el huésped se debilite. Sus defensas perderán valor y se hará más susceptible al parásito. Una segunda condición estará determinada por el número y fortaleza de las unidades infecciosas.

En vista de que los tubérculos de patata en activa germinación han demostrado ser muy resistentes a altas concentraciones de inóculo, en tanto no pierdan ese carácter activo, la debilidad del huésped se ha logrado mediante un método basado en el uso de tubérculos pelados y libres de sus puntos germinativos. De esta forma se ha podido medir la susceptibilidad de los tejidos vivos del tubérculo (sin otras circunstancias modificativas) frente a diversos tipos de inóculo. Se prestó especial atención al incremento de tejido putrefacto, según el número de celulas iniciales del inóculo y se realizaron experiencias pilotos sobre el efecto del estado de nutrición y edad de los cultivos bacterianos utilizados.

Los resultados son concluyentes en mostrar que cuando los tejidos de la patata son inoculados con cultivos de *E. carotovora*, var. *aroideae*, las diferencias en el incremento de tejido putrefacto se suelen encontrar en los primeros estados de la infección. Cuando los cultivos utilizados como inóculo se desarrollan o mantienen en condiciones desfavorables para la bacteria, el inóculo inicia su actividad después de cierto tiempo de aparente letargo o período de incubación, a menos que esté muy inactivado. Estos resultados parecen demostrar que el efecto bacteriostático no se debe atribuir a un fallo del potencial de inóculo.

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CYTOARJESIS IN POTATO TUBERS

III. Susceptibility of living potato tissues to soft-rot: Effects of humidity and temperature *

BY

ROMAN VICENTE JORDANA

The third condition quoted by Garrett (1956) for occurrence of disease (that is, occurrence of suitable environmental conditions for penetration and infection of the host by the parasite) was considered to study the susceptibility of living potato tissues to soft-rot under the influence of temperature and humidity. These factors also proved in previous work to be critical for a better understanding of the bacteriostatic effect. This study was linked to that on the effect of inoculum potential (Vicente Jordana, 1958b) and the same method involving peeled tubers was used.

A) EFFECT OF HUMIDITY

1. Importance of humidity in the development of soft-rot and host reaction.

Under the supposition that special defence mechanisms operate in arresting infection of potato tubers, humidity is one of the most important factors to be studied, because it plays a role with both parasite and host.

The folloving points are of interest:

a) Breakdown of potato parenchyma by bacteria requires a certain amount of free moisture, otherwise bacterial infection is stopped. Fungal mycelia often grow easily at lower humidities than those at which bacteria do.

(*) The author is indebted to Dr. J. Rishbeth of the Botany School, University of Cambridge, for his critical advise and helpful suggestions.

b) The reaction of the host is linked to humidity in two ways. One is connected with the formation of wound-periderm and the other, since humidity affects the germinative processes, is related to the bacteriostatic effect itself.

a) Humidity in the breakdown of potato tissues.

(1) *Humidity requirements.*

Low humidities have the effect of arresting soft-rot (Brierley, 1928; Rudd Jones, 1948). According to Rudd Jones, at relative humidities of 90 % and below rot is completely stopped at 25° C. for tubers of the variety Majestic, although it might be expected to vary with other varieties. Secondary bacterial rots may develop on blight-infected tubers after a fortnight at an R.H. of 100 and 97.5 %. At an R.H. of 89.9 and 94.8 % blight-infected tubers showed various saprophytic fungi growing on the surface. At still lower humidities there was no indication of secondary infection.

The study carried out by Brierley with four bacterial strains showed that at 22° C. *Bs. mesentericus* remained active at 80 %R.H. and at 12° C. *E. phytophthora* was active at 87 % R.H. *E. aroideae* started rot at 22° C. in the majority of inoculated tubers, but, like *E. carotovora,* was no longer active in any at the end of the test (19 days).

Experiments with other plants show that bacterial rots always require a high relative humidity (Volcani and Wahl, 1954) or the presence of free water (Sabet, 1954).

(2) *Water content of tuber.*

Water content of the tuber seems also to be important in the development of infections in potato tubers. When the water content of the tuber is increased either by soaking or by injection the tuber becomes more susceptible to *Botrytis cinerea, Fusarium fructigenum, Rhizopus nigricans* and *Phytophthora erythroseptica* (Chattopadhyay, 1947) and is rotted more rapidly by *E. carotovora* (Gregg, 1948). According to Tolba (1953), injections with water render potato tubers more susceptible to *E. aroideae* and its enzyme and less susceptible to *E. phytophthora,*

although they do not affect the action of the *E. phytophthora* enzyme.

Rudd Jones (1948), after studying the water content in inoculated potato tubers of three varieties, stored for a period of two months, concluded that water content does not limit the host reaction but does actively limit spread of the bacteria and thereby allows the host reaction to take place.

b) Influence of temperature and humidity on host reactions.

(1) *Wound-periderm formation and its breakdown by bacteria.*

Humidity and temperature seem to have and influence on the production of the cork-barrier as a reaction to wounding. According to Artschwager (1927) suberization takes place in 24 hrs. at temperatures between 21 and 35° C. and at 94 % R.H. Rudd Jones (1948) and Rudd Jones and Dowson (1950), using inoculated tubers, found that at temperatures above 20° C. and in a saturated atmosphere bacteria spread so rapidly that the host cells are killed before suberization commences. Suberization seems to be favoured by low temperatures (5-10° C.) and an R. H. of 86 %. When inoculated tubers were subjected to a temperature of 25° C., following 3 days at 5-10° C., bacteria penetrated that barrier and produced a further rot. The authors concluded that low temperatures and humidities favour formation of the retarding barrier, while on the other hand high temperatures favour multiplication of bacteria.

Smith and Smart (1955), experimenting on relationships of soft-rot development to protective barriers, found that slices kept in moist chambers for two or three days at 70-80° F. (21.1-26.6° C.) before inoculation developed much less decay than those held for one day. Suberin and periderm production was more extensive at those temperatures. At 50-60° F. (10-15.5° C.) the amount of periderm developed in four days was more or less similar to that formed in two days at the higher temperatures. At 40° F. (4.4° C.) no periderm developed in four days.

Formation of periderm was observed during the present experiments and suberized cells were detected by the method of Stoughton (1930) (thionin and orange G) in previous work (Vicente Jordana, 1955).

(2) *Influence of the above factors in germinative activity.*

Since humidity has a great effect on soft-rot and it seems not greatly to affect soft-rot in active germinated tubers, its influence in germination must also be considered. In fact, moist conditions and optimal temperatures favour germination, and high humidities give a better development of buds than low ones. Therefore, a study of the effect of humidity on inactive tubers or on their parenchyma is of importance to distinguish between what may be called mechanical or pasive defences and the influence of germinative activity in the arrestment of infection.

2. Experiments on the effect of humidity on the skinless parenchyma of potato tubers.

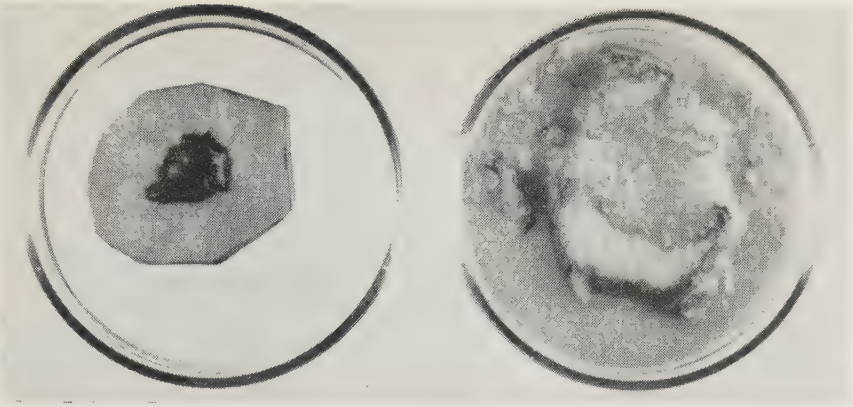
a) On potato slices.

The effect of humidity on the rotting of potato slices has been observed an unrecorded number of times because this was for a long period the usual method of checking the effectiveness of inocula. All varieties used in the present and former work have been checked in this way.

As is well known to plant pathologists, potato slices obtained by peeling and cutting aseptic tubers, placed in Petri dishes and then inoculated with bacterial cultures, are macerated rapidly in two, three, or four days at suitable temperatures in the presence of free water. In dry conditions decay develops slowly and quite often stops. Rotting also occurs when water condenses on the under surface of the lid and drops on to the slices. Plate no. 1 shows the difference in development of soft-rot between two potato slices of Ulster Supreme variety held in dry and wet conditions respectively at 25° C., 48 hrs. after inoculation with *E. carotovora,* var. *aroideae.*

b) Formation and breakdown of wound periderm.

No systematic study of the formation of new periderm was carried out, but it was found that *wound periderm was produced in peeled tubers in dry environmental and in relatively humid conditions. Breakdown*



Dry 25° C. 48 h. Water

PLATE 1.

Effect of free moisture on inoculated slices of peeled potato tubers of variety Ulster Supreme, showing the greater extent of rotting of the slice kept in water

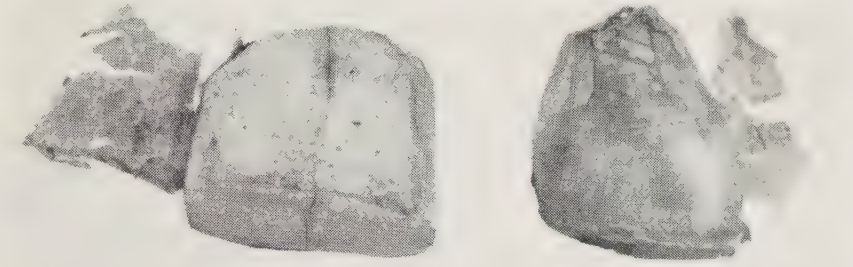


PLATE 2.

Periderm development in uninoculated peeled potato tubers left at room temperature for two months and in unsterile conditions. The wound periderm has been peeled back to show it more clearly. var. Sharpe's Express.

PLATE 3.

Surface contamination by fungi on peeled tubers of variety Sharpe's Express, which were inoculated with *Erwinia carotovora*, var. *aroideae* and kept at 100 % R. H. at 22.5° C. in glass-stoppered bottles for 18 days.

of periderm and development of soft-rot required the presence of free water.

The following experiments were performed:

(1) *Study of natural infection under dry conditions.*

Formation of wound periderm and the incidence of natural infection, with the possible development of soft-rot, were studied in half-tubers of four varieties: Sharpe's Express, Catriona, Eclipse and Arran Pilot. 15 tubers of each variety were peeled and cut into two, the pieces being piled up in crystallizing dishes and left for two months at room temperature. No attempt was made to keep the tubers free from contamination. The results are shown in table no. 1. Periderm developed in all

TABLE 1.

Incidence of infection and formation of new wound periderm in uninoculated peeled pieces of potato tubers, after two months at room temperature (17-20° C.) in dry conditions.

VARIETY	No. of pieces	% Formation of new periderm	MICROBIAL POPULATION		
			% Internal infections spp. (origin unknown)	Superficial develp. of fungi	Soft-rot
Sharpe's Express...	30	100	40	+	—
Catriona.....	30	100	53	+	—
Eclipse.....	30	100	30	+	—
Arran Pilot.....	30	100	43	+	—

the pieces, as seen for example in plate no. 2 showing a piece of Sharpe's Express variety. Surface development of fungi was also general, although some pieces which appeared to be very dry were less contaminated. The percentage incidence of infection was different for each variety. Soft-rot was not produced.

The amount of water in peripheral and central tissues is shown in table no. 2.

TABLE 2.

Percentage water content in healthy tissues of four peeled tubers of Sharpe's Express variety, after two months in dry conditions at room temperature.

CENTRAL TISSUE		PERIPHERAL TISSUE	
Single values	Mean	Single values	Mean
73.7	74.1	71.0	67.8
75.5	—	76.4	—
70.9	—	54.8	—
76.1	—	68.8	—

(2) *Study of experimental soft-rot under moist conditions.*

10 peeled tubers of Sharpe's Express variety were inoculated by method (1) (Vicente Jordana, 1954, 1958a) with *E. carotovora,* var. *aroideae* and placed on tripods in glass-stoppered bottles. The bottles contained a certain amount of water at the bottom. After incubation at 22.5° C., soft-rot developed rapidly but later was limited to some extent, although in some tubers it was not completely stopped. After 18 days the whole surface of the tubers was covered by fungal mycelium (plate no. 3). Periderm was formed by this time, but its separation from parenchyma tissues was more difficult than that of periderm produced in dry environmental conditions after two months.

By increasing the temperature, the relative humidity of the atmosphere was raised. Tubers of Sharpe's Express, Majestic and King Edward, prepared in the above manner and incubated at 35-37° C., were attacked rapidly and became a mass of decaying matter within a few days.

(3) *Development of experimental soft-rot in the presence of free moisture.*

(a) *Partially immersed in water:*

1*st. Freshly peeled tubers.*—Freshly peeled tubers partially immersed in water were rotted to a considerable extent a short time

after direct inoculation or contamination of the water with a bacterial culture. The attack follows a similar course to that in potato slices, but sometimes in portions exposed to the air rotting takes place slowly and wound periderm develops. Frequently with large tubers it requires a long time before one hundred per cent of the tissue is rotted. Otherwise new periderm, although it may be contaminated by fungi, has the appearance of unrotted material. Disruption of the internal tissues is seen by scratching the surface or probing with a scalpel or punch. Nevertheless, wound periderm was more sensitive to soft-rot than normal skin of tubers when in contact with water. Normal skin is unattackable by soft-rot bacteria.

2nd. Experimental soft-rot with one week-old wound periderm on peeled tubers. — The breakdown of tissues in whole inactive tubers leads to the supposition that wound periderm formed in peeled ones is an insufficient protection to arrest soft-rot development. Nevertheless, an experiment was performed with tubers of the variety Majestic and observation of breakdown of tissues was made in several other varieties. According to McKee (1954), resistance to experimental infection with *Fusarium caeruleum* and *F. avenaceum,* was greater when inoculation took place after some time after wounding than when wounds were inoculated immediately.

Ten tubers of the variety Majestic were peeled and placed on tripods in glass-sotppered bottles containing water at the bottom. Five of the tubers were prepared for inoculation and in each a hole was made with a cork-borer (method 2, Vicente Jordana, 1958a) to permit the formation of periderm there (series a). The other five were wounded with the same instrument at the time of inoculation (series b). After eight days at 22.5° C. inoculations were made by depositing into the holes a loop of a 48-hour culture of *E. carotovora,* var. *aroideae,* grown on a solid medium used for standard inocula.

Tubers were placed in water and measurements of the depth and external width of lesions were first taken after 24 hrs. At this time the increase in depth of lesions was noticeable in all tubers. Increase of external width was very slow, there being practically none for series (a) after 3 days, with the exception of one tuber which gave 10.5-11 mm. and 14-15.5 mm. after 24 and 48 hrs. respectively. The mean width after 48 hrs. for the other four tubers of this series

was 3.8 mm. For series (b) the average width after 48 hrs. was 5.6 mm.

Seven days after inoculation, soft-rot in both series extended all over the surface of the tubers in contact with water and it was also widespread on the sides of the tubers above water level. Width of lesions and fresh weight were highest for series (b) and depth for series (a) (table no. 3).

TABLE 3.

Effect of humidity.—Increase of soft-rot in peeled tubers of variety Majestic, inoculated with *E. carotovora* var. *aroideae*, 8 days after their preparation. Depth was measured by inserting a punch.

	DEPTH (mm.)			AFTER 7 DAYS		
	At inoculation	24 hrs.	48 hrs.	Depth (mm.)	E. W. (mm.)	Fresh weight (g)
8 days-old periderm (series a).....	6.4	8.1	9.7	15.2	38.7	22.6
Freshly wounded (series b).....	5.4	6.9	7.9	12	58.9	27.6

E. W.: External width.

From these results it is believed that wound periderm may effectively protect potato tissues from soft-rot at some stages of infection, but it is not enough to stop development of bacteria in the absence of other mechanisms of defence, as has been suggested earlier (Vicente Jordana, 1955 (*); Stapp and Hartwich, 1956).

(*) A quotation from the Spanish version (1955):

"These studies have led us to consider the value of the barrier of suberized cells and, in effect, it has been possible to prove, following the differential techniques (thionin and orange G) of Stoughton (1930), that the inoculated line of lesion of the bacteriostatically active tubers clearly showed the presence of these cells. In spite of this reason, the experiments which have been pointed out must be taken into account as important, because even if the defence of the tuber were due to these cells, it cannot be doubted that cellular proliferation or germinative activity must play an important part in the suberization of the cells.

Various questions, however, remain to be asked about the effectiveness of the wall of suberized cells. If we consider that Artschwager found that suberization was produced up to 35° C. and a degree of humidity of 94 %, it is not easy to understand that at this temperature and in a saturated atmosphere the number of rotted tubers should reach 100 %. In the same way, it is to be supposed that those tubers which have been for a month at temperatures of 18° to 25° C., and which have had time enough to form their resistant dermis, would naturally retain this protection even when germination was arrested. But this does not happen. On the contrary, they rot just as easily as those in which germination was arrested when

(b) *Increase of experimental soft-rot in pumice powder with various moisture contents:*

64 small tubers of Sharpe's Express variety were peeled, and inoculated by method (2) with a standard 18 hrs. inoculum. After inoculation tubers were divided into two series of five and three units for measurements after 18 hrs. and three days respectively. Tubers were placed over thick layers of pumice powder at various moisture contents, with the lesion facing downwards towards the filter paper. For each moisture content, both series (a total of 8 tubers) were placed in the same container. For the lowest one the filter paper was slightly wet and was wrung out before use. Tests were also made under dry conditions and with tubers standing in water: for the former the lesion faced upwards and for the latter tubers were enveloped in muslin. The moisture content of the pumice powder was determined by measuring the loss of weight after two days at 100° C. A third series of four tubers for measurements after 20 days was prepared later. Tubers were covered by muslin to keep the surface moist.

The extent of infection at the different moisture contents is shown

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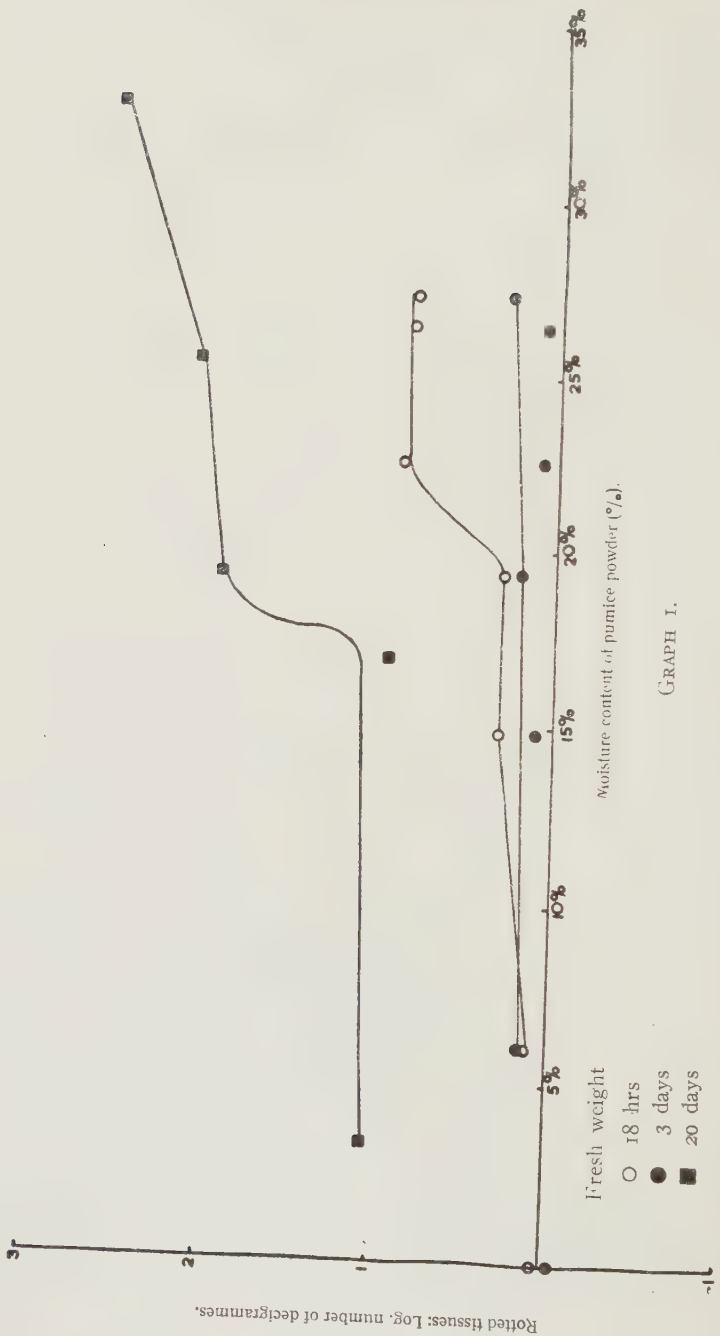
they were only recently inoculated. If one can accept that tubers submitted to 37° C. for a few days and then passed on to other lower temperatures could form their resistant dermis, this is not easy to believe for those in the previous example, since their dermis was already formed. On the other hand we have the experiments of Rudd Jones and Dowson which, as we have already seen, establish the optimun conditions for suberization at relatively low temperatures and point out the temperature range 20° - 25° C. as the best for the development of micro-organisms. These, in fact, are the temperatures at which the phenomenon of bacteriostatic activity is most forcibly evident."

"We could make another observation about suberization. If germination, or, to be more precise, germinative activity has a direct influence on the formation of suberized cells, we should have to conclude that these cells are in a continous state of formation and destruction, since when the germinative process is arrested by temperature the barrier of those cells in which it had already been formed is easily cleared by micro-organisms. If this is so, we could perhaps presume that the idea of suberization as the sole defence system is insufficient to explain resistance against micro-organisms, but that is possibly no more than a secondary system, an auxiliary of the true system of defence. There must surely be something else, originating in cellular activity itself, which gradually destroys the germs which clear that barrier."

At the present time other experiments have proved that not only temperature but also other systems of inactivation, like those with peeled tubers reported in this paper, may favour soft-rot in spite of suberized cells. What it seems of interest from experiments with peeled tubers is that suberization do not require the direct influence of growing buds and that resting activity in cells is enough to follow the processe.



GRAPH 1.

Susceptibility of potato tissue to soft-rot.
Effect of humidity on peeled tubers of potato variety Sharp's Express. Inoculum: *E. carotovora*, var. *avoidae*.

in graph no. 1. Complementary data are collected in tables no. 4 and no. 5. The fresh weight of the whole amount of soft-rot at 100 % humidity after 3 days was 6.85 g. (mean of 3 tubers). For dry conditions (same mean and time) the fresh weight was 0.11 g.

TABLE 4.

Effect of humidity.—Complementary data on the increase of soft-rot in peeled potato tubers of Sharpe's Express variety inoculated with *E. carotovora,* var. *aroideae.*

% Water content in pumice powder (after 10 days)	SIZE OF LESIONS (mm.)						% Water content in rotted material	
	E. W.		I. W.		D.			
	18 hrs. (a) x	3 days (b) x	18 hrs. (a)	3 days (b)	18 hrs. (a)	3 days (b)	18 hrs. (a)	3 days (b)
0.4	5.1	10 (c)	5.0	6.2	6.6	7.3	76.2	64.7 (P)
6.1	5.5	6.0	5.1	6.5	7.0	7.3	73.4	67.2 (P)
14.9	6.0	5.8	5.4	6.5	7.4	8.3	76.2	75.2 (P)
19.4	6.4	7.8	7.0	6.7	7.2	8.0	76.1	70.9 (P)
22.6	5.6	13.3 (d)	6.4	8.0	7.2	9.0	72.7	75.5
26.4	6.1	9.3	6.6	8.0	7.2	8.5	74.3	78.6
27.3	6.8	11.0	7.0	8.0	7.5	8.0	73.5	78.6
100.0	6.2	—	5.1	9.5	7.5	9.3	73.4	80.7

E. W.: External width. — I. W.: Internal width. — D.: Depth. - (a) Means of 5 tubers. With x average from 10 measurements. - (b) Means from 3 tubers. With x average from 6 measurements. - (c) Two radial extents of 3 and 3.5 mm. (d) One radial extent of 4 mm. - (P) New periderm formed.

TABLE 5.

Effect of humidity.—Complementary data on increase of soft-rot in peeled potato tubers of Sharpe's Express variety inoculated with *E. carotovora,* var. *aroideae,* after twenty days. Means from four tubers.

% Water content in pumice powder (after 20 days)	SIZE OF LESIONS (mm.)		% Water content in rotted material	Observations
	I. W.	D.		
Dry conditions.	3.8	5.4	—	Uninoculated. Not in pumice powder.
3.5	9.3	8.5	84.8	
17.1	7.3	8.4	85.7	
19.4	15.6	13.4	84.9	
25.5	27.3	21.3	87.0	
32.8	V. E.	T.	84.0	

V. E.: Very extensive.—T.: Complete.

Secondary infection mainly produced by fungi was developed after twenty days, especially with tubers held over pumice at a moisture content of 19.4 %, 25.5 % and 32.8 %. Other tubers also showed contamination. The individual behaviour of tubers must be considered as well as the conditions within a given container and means are therefore of relative value in expressing the extent of soft-rot. Variations in residual activity of the tuber or in the conditions of humidity may give great differences at early stages of infection. Table no. 6 gives some of the differences obtained between single observations.

TABLE 6.

Effect of humidity.—Some differences in the extent of soft-rot in single peeled tubers held in the same container: (a), in free water at an early stage of infection, and (b), after 20 days over pumice powder with a much lower moisture content.

WATER	TIME	SIZE OF LESIONS (mm.)			ROTTED MATERIAL	
		E. W.	I. W.	D.	Fresh weight (g.)	Content of water (%)
100 % (free water).	3 days.	7-21	5.5	8	1.98	81.8
—	—	15-17	9.0	10.0	5.92	82.7
—	—	V. E.	14.0	10.0	12.65	79.7
17.1 % (P. P.)	20 days.	—	14.0	13.0	2.25 (2)	85.4
—	—	—	5.0	6.0	0.08 (mean) (2)	82.2 (mean)
—	—	—	5.0	8.0		
—	—	V. E. (1)	5.0	6.5	1.32	86.7

(1) Secondary infection.—(2) Slight contamination.—(P. P.) Pumice powder.

3. Discussion.

These last results together with those obtained at R.H. or in dry environmental conditions seem to prove that a relatively high content of free water is required for soft-rot production. Soft-rot steadily increases with the amount of water. By looking at the tables (no. 4, no. 5 and no. 6) it can be seen that the water content of the soft-rotted tissues was in many instances (especially after 18 hrs. and 20 days) the same or similar for tubers kept at several degrees of moisture, while the extent of rot showed great differences (table no. 5).

It may also be considered that when soft-rot appears in drier conditions, its increase may be due to the freeing of the water content of the tissues by external factors (as for instance, breakdown of cell walls by preassure, hits or bruises) or by bacterial activity, together with the production of water by bacterial metabolism. It is thought that the intercellular water content of the tissues has no effect on the quick progress of soft-rot before it is freed. Once bacterial activity starts, progressive increase of water from the disrupted tissues can help the development of bacterial progeny to produce the progresive increase of soft-rotted tissues. Measurements of the water content of tissues proved that they have a mean of about 75 %, but as little as 25.5-33 % of water in pumice powder may help to produce a considerable or complete rot (table no. 5).

4. Conclusions.

From the above experiments on the effect of humidity on the development of soft-rot in potato parenchyma it may be concluded:

1. The extent of soft-rot and associated infections developing in peeled potato tubers is a function of time and humidity.
2. With low humidities soft-rot may be arrested but other types of infection develop to some extent.
3. The degree of humidity associated with at least 20 % moisture content in pumice powder seems necessary for increase of soft-rot. At lower humidities infection develops slowly.
4. Formation of wound periderm seems inadequate to arrest the development of infection or to prevent contaminations on the surface of peeled tubers.
5. Wound periderm is more sensitive than normal skin of tubers which is unattackable by soft-rot bacteria.
6. It may also be concluded that the intercellular water content of the tissues has no effect in many instances on the quick progress of soft-rot unless it is freed by bacterial activity or as a result of external factors.

B) EFFECT OF TEMPERATURE

1. Importance of temperature in rotting of potatoes.

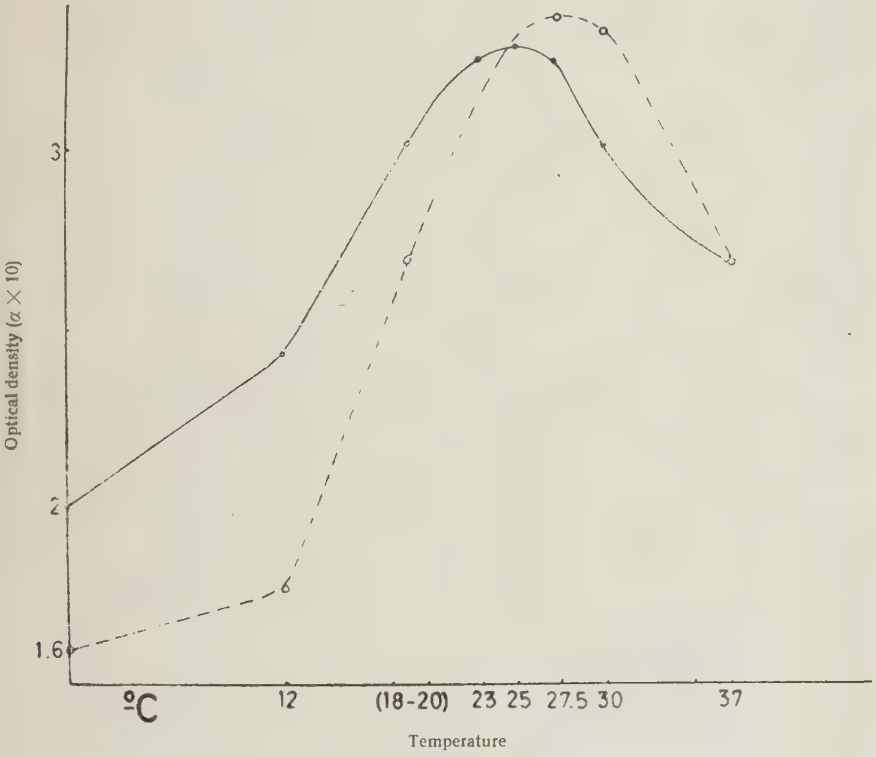
The effect of temperature in potato rots is in many ways closely connected with that of humidity and depends on the activity of the parasite as well as that of the host. Knowledge of the effect of temperature on inoculated peeled tubers was mainly interesting in so far as it permitted choice of a temperature at which the activity of the bacteria in the tissues would be reduced without complete loss of effectiveness (in order to help the activity of the host).

According to Bergey's Manual (1948) and Elliot (1951) the optimum temperature for *Erwinia carotovora* is 25 - 30° C. and for *E. aroideae* 35° C. As judged by the depth of rot produced in inactive potato tubers, the strain used in the present experiments grew best over the range 22.5° to 30° C. Growth was also colorimetrically determined on nutrient (meat infusion + glucose). In this procedure, the growth of *E. carotovora,* var. *aroideae,* at various temperatures was measured in terms of optical density increase, determined in an ETCO photoelectric colorimeter (mod. 308B) using a green filter. The apparatus was calibrated with distilled water. The results obtained from parallel cultures (5 ml.) after 24 hours are expressed in graph no. 2. These results together with other obtained through the experiments seem to indicate that the optimum growth temperature of the variety referred to above is found about 25-27° C.

Studies on the effect of temperature on soft-rot showed that the amount of decay increases steadily from about 10° C. to 37° C. (Paine, 1917; Paine and Chaudhuri, 1923; and Rudd Jones, 1948). Brierley (1928) found that while *E. aroideae* and *Bs. mesentericus* developed normal rot at 37° C. *E. carotovora* showed only a trace of rot and *E. phytophthora* produced none, or only incipient rot. Rudd Jones (loc. cit.) considered it possible that these results were due to o drying-out of the inoculum.

A pretreatment of the tubers at 37° C. allows a more rapid spread of *E. carotovora* (Gregg, 1948). In dry atmospheres tubers inoculated with *E. aroideae* may become rotted to an extent of some 50 to 80 %

at 34.5° C. after 45 days, whilst tubers in contact with water were rotted to an extent of 83.3 to 100 % in 30 days at this temperature and to 100 % in less than 30 days at 37° C. Well germinated tubers, either placed in water or held in a dry atmosphere, after inoculation, may remain



Graph 2.

Growth of pure cultures of *E. carotovora*, var. *aroideae*, on nutrient (meat infusión + glucose) at various temperatures, after 24 hours. Measurements in terms of optical density (α) increase are expressed as colorimeter units.

sound with a little increase of soft-rot or affected tissues after 30 days or more at temperatures of 18-25° C. At such temperatures tubers germinate actively. Tubers kept at temperatures over 33-34° C. do not develop buds (Vicente Jordana, 1955).

2. The effect of temperature on peeled potato tubers.

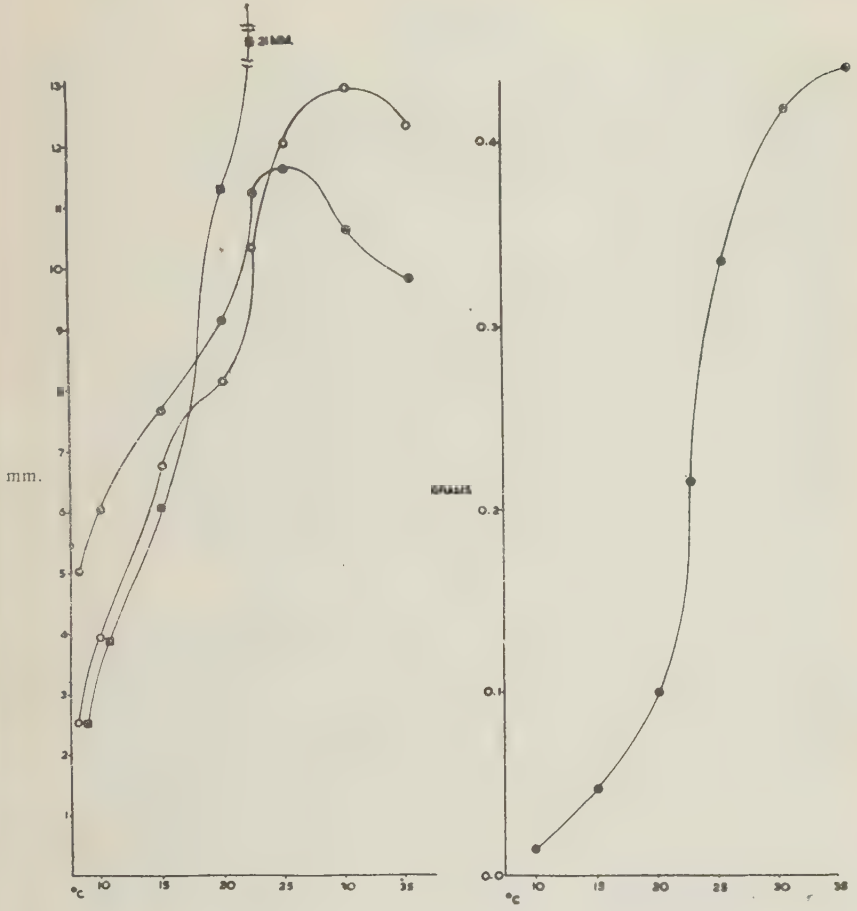
One experiment was performed in order to ascertain the effectiveness of the inoculum at different temperatures and the incidence of contamination by other organisms under the conditions used in the experiments. Special interest was paid to observing the activity of the inoculum at 22.5° C. at which most of the work was done. 35 peeled tubers of Sharpe's Express variety were inoculated with a 0.1 dilution of a suspension of standard inoculum of *Erwinia carotovora,* var. *aroideae* having a plate count of about 5×10^9 cells per ml. After tubers were wrapped in wet muslin they were placed over pumice powder with a moisture content of about 40 %, held in china containers. Five tubers were used at each of the following temperatures: 10, 15, 20, 22,5, 25, 30 and 35° C.

The results, obtained after 48 hrs., are expressed in graph no. 3a, 3b. Some differences are found between these results and those reported above, obtained from whole tubers. Rot increased steadily with temperature, optimal conditions for rot being obtained between 25-35° C.

The average water content of the rotted tissue was higher at 22.5 and 25° C. than at other temperatures. At 35° C. rot extended all round the tubers. It was found in humidity tests (p. 44) that high temperatures favour decay of peeled tubers in a moist atmosphere. The fact that tubers at 35° C. were slightly contaminated on the surface and that two of them showed gas production must be also taken into consideration. Contamination was observed in one other tuber at 30° C., but none of the remaining 29 tubers of the experiment showed symptoms of secondary infection.

Since contamination and humidity may affect the extent of rot, the results expressed in graph. no. 3a, compared with those in graph. no. 2, seem to confirm the idea referred before that to obtain comparable estimates of the activity of a pure inoculum, depth of the lesions may be taken as the most reliable measure (Vicente Jordana, 1958b).

Temperatures of 20-22.5° C. seem to be the most suitable for experiments on the bacteriostatic effect. When tubers are in active germination, the bacteriostatic effect is clearly shown between 20-25° C.



(a) (b)

● Depth of lesion.
○ Internal diameter of lesion.
■ External width of lesion.

● Dry weight of rotted material.

GRAPH 3.

Susceptibility of potato tissue to soft-rot.

Effect of temperature on peeled tubers of potato variety Sharpe's Express 48 hours after inoculation.
Inoculum: *E. carotovora,* var. *aroideae* (5.4×10^9 organisms in the suspensión/ml.).

SUMMARY

The susceptibility of potato tissues to soft-rot, under the influence of humidity and temperature, was studied using a method involving peeled tubers. These experiments were also considered as an attempt to determine the activity for defence of living tissues freed from the influence of buds.

Results seem to prove conclusively that potato tissues are sensitive to the development of soft-rot and other infections when they are not under the influence of growing buds. Free moisture is an important factor for development of soft-rot: the extent of rot and associated infection in peeled potato tubers is a function of time and humidity. With low humidities soft-rot may be arrested but other types of infection develop to some extent. From this it may be concluded that the intercellular water content of the tissues has no effect in many instances on the quick progress of soft-rot unless it is freed or produced by bacterial metabolism or as a result of external factors.

Formation of wound periderm seems also inadequate to arrest absolutely the development of infection or to prevent contaminations on the surface of peeled tubers. Wound periderm is more sensitive than the normal skin of tubers which is unattackable by soft-rot bacteria.

Soft-rot develops over a wide range of temperature (20-35° C.).

Since active tubers are not greatly affected by free water and temperatures of 18-25° C., these results may confirm the critical value of the effect of humidity and temperature of a better understanding of the bacteriostatic effect.

RESUMEN

En este trabajo se expone un estudio experimental del valor de la temperatura y la humedad en la susceptibilidad a la putrefacción de tubérculos pelados de patata. Tiene por objeto, en primer lugar, aplicar la tercera de las condiciones expuestas por Garrett (1956) como indispensables para que se produzca la infección, es decir, considerar la concurrencia de ciertos estados ambientales favorables para la penetración del parásito e

infección del huésped. En segundo término, se ha querido determinar la actividad de defensa de los tejidos vivos del tubérculo, libres del influjo de los brotes activos, esperando con ello precisar la diferencia existente entre el comportamiento de los tubérculos activos y los inactivos o inactivados experimentalmente, bajo condiciones variables de humedad y temperatura. Como ya se expuso en otros trabajos, los tubérculos activos mantienen la bacteriostasia natural aunque estén parcialmente sumergidos en agua y sometidos a una temperatura comprendida entre los 18-25° C.

Los resultados obtenidos demuestran que para lograr la putrefacción blanda de los tejidos de patata es necesaria la presencia de agua libre. Humedades relativas del ambiente no son tan efectivas, aunque pueden favorecer el desarrollo de otras enfermedades. Igualmente y contrariamente a lo supuesto por algunos autores, parece que el contenido de agua intracelular no tiene gran importancia para el progreso rápido de la infección en gran número de casos, a menos que sea liberada por actividad bacteriana o como consecuencia de factores externos.

Se confirma también la opinión de que la formación de peridermo no parece suficiente para paralizar totalmente la infección, ni para evitar la contaminación de la superficie de los tubérculos pelados. Este peridermo es más sensible a la actividad bacteriana que la piel normal del tubérculo, que es inatacable por las bacterias de la putrefacción blanda.

Finalmente, se ha podido ver que la putrefacción es activa en una amplia escala de temperatura (20-35° C.).

Estos resultados, en especial los relativos a la humedad, dan un valor crítico para una mejor comprensión del efecto bacteriostático y demuestran que el proceso de suberización puede desarrollarse sin la influencia directa de los brotes en crecimiento.

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INSTITUTO «JAIME FERRAN», DE MICROBIOLOGIA

ESTUDIO MICROBIOLOGICO DE ALGUNAS CARNES DE JABALI Y VENADO

POR
EMILIO RONDA LAIN

Aparte de las publicaciones de Kelser (17), Verge y Thieulin (38), Nevot (27) y otros, que se ocupan de la importancia y oportunidad de las investigaciones bacteriológicas sobre microorganismos contaminantes de carnes, y las constituídas por tratados especializados en materia alimenticia, [Casares (5), Sanz Egaña (31), Lafenetre y Dedieu (19), Tanner (37) bis,] existen otras consultadas por nosotros, que si bien pertenecen a otras especies animales diferentes de las aquí estudiadas, al menos servirán de antecedentes —por similitud de origen— a los datos que nosotros vamos a encontrar, reflejando al mismo tiempo una más amplia visión del tema: Zbeigniew (40), haciendo recuentos bacterianos totales, en muestras de carne de vacuno, una hora después del sacrificio, encontró que todas estaban más o menos contaminadas: en una proporción de un 64 % de muy contaminadas; asímismo (también sobre carne de vacuno) Ayres y Adams (1), valoran la población microbiana por ellos detectada, en 100.000 a 10.000.000 de células bacterianas por cm.^{2} y la relación de aerobios anaerobios era de 6 : 1; mientras que Kirsch y cols. (18), en sus recuentos sobre placa encuentran de 1.400.000 a 95.000.000/gr. con predominio de *Pseudómonas* y *Achromobacter*, algunos cocos y lactobacilos; y Muller (25), aisla en muestras de carne de cerdo algunas salmonelas.

Parece ser, que la población bacteriana de carnes está integrada, según los diversos autores, por especies de diferentes géneros: *a) Micrócoccus pyogenes, M. sulflavescens, M. ureae, M. nitrificans, M. percitreus, M. flavescens* y *Streptococcus del grupo* D; *b) Salmonella typhi, S. paratyphi,* A y B, *S. cholera suis, S. anatus,* especies de *Proteus, Alcaligenes amoniagenes* y otras especies de *Alcaligenes, Escheri-*

chia coli y otros; *c)* diferentes especies de *Pseudomonas psicrofilos* y *Achromobacter, Flavobacterium anteniforme, F. sulfureum* y otros *Flavobacterium; d)* especies de lactobacilos y otras bacterias lácticas; *e)* otras diferentes especies detectadas en casos muy particulares, como el *Bacterium phosphorescens* y varias especies de *Photobacterium (Pflageri, physceri, Balticum, Indicum y luminosum); f)* diferentes especies de bacterias esporuladas *(Bacillus y Clostridium).* Estas especies han sido estudiadas y experimentadas por diversas causas o con distintos fines.

Por cuanto a esporulados se refiere, hay predominio de publicaciones en la literatura científica de inspección de carnes: Moundt y Kitchen (23) identifican varias especies de *Clostridium bifermentans, mucosum, sporógenes, parabifermentans, septicum, paraputridum* y *putrefaciens)* en jamones moteados; Steinkraus (34), determina el grado de contaminación por bacterias anaerobias putrefactivas en carnes de vaca y cerdo, y comprueba que tan solo se produce una acción efectiva esporoestática cuando se conjugan los efectos de azúcar, nitrato y nitrito; mientras que Schonberg (32), comprueba el efecto del contenido bacteriano de los condimentos que puede aumentar la contaminación de productos cárneos en especies esporuladas, sobre todo del grupo *mesentéricus-subtilis;* por último, Leistner (20), estudia la presencia de anaerobios, especialmente del *Cl. perfringens* en carnes frescas y conservadas, examinando las contaminaciones en este último caso (conservas de jamón, por ejemplo) pueden ser provocadas por la gelatina añadida.

Por otra parte, es interesante destacar la intervención que las bacterias halófilas pueden tener en los productos cárnicos, de ello son buena prueba los trabajos de Muller (26), que advierten de que la presencia de elevadas concentraciones de sal no bastan para prevenir el desarrollo bacteriano, ya que no sólo existen ciertas bacterias patógenas y saprofitas sal-resistentes, sino que también él ha encontrado gérmenes halofílicos que producen manchas rojas en la carne.

Muggerburg (24) y Coretti (6) señalan, también, a las especias y otros aditivos como vectores de contaminación bacteriana en carnes.

En cuanto a los factores que intervienen en la supervivencia de la flora bacteriana de las carnes, diremos que los trabajos de Steinke y Foster (33), acerca del efecto de la temperatura y el almacenamiento sobre cambios microbianos en alimentos cárnicos, han sido varios los

autores que hacen publicaciones a este respecto: Ice y cols, (12), comprueban el efecto del calentamiento sobre las cifras de recuento bacteriano; Ingrams y Hobbs (14) realizan exámenes habituales sobre 175 muestras de jamón que se habían sometido a pasteurización, observando que sobrevivieron algunas especies de *Clostridium*, estreptococos *pseudomonas* y micrococos; también Tanikawa y Tezuka (36) se interesan por la relación entre el grado de esterilización y la concentración de esporas bacterianas, a la vez que en colaboración con Wenohi estudian los cambios bacteriológicos que se producen por bacterias similares al *B. megatherium* y al *B. mesenthericus vulgatus*, por el calentamiento a distintas temperaturas durante diferentes intervalos de tiempo. Mientras Mc. Clesky y Boyd (22), al ocuparse de la longevidad de enterococos y coliformes en carnes sometidas a bajas temperaturas, comprueban que temperaturas de 5° a 8°C. detienen el desarrollo de los primeros, en tanto que el número de coliformes aumenta cien veces en unos diez días, aproximadamente; Huttchins y cols. (11), estudiando la viabilidad del *Br. melitensis* en jamones curados, que estaban infectados naturalmente, observan que éstos resisten durante 21 días. Callo e Ingram (4), hacen consideraciones y discuten sobre el origen, localización y modo de difusión de las bacterias verdes de la carne, así como también el efecto del pH y temperatura sobre los tejidos y por consiguiente sobre el desarrollo bacteriano.

Por otra parte, Ingram (13), en su trabajo sobre bacteriología de las carnes semi-conservadas, estudia algunos factores que pudieran intervenir en la presencia de determinadas especies, particularmente asociaciones psicrófilas *(Achromobacter* y *Pseudomonas)*; este autor comprueba la afinidad de las bacterias por diferentes productos; así: *a)* Micrococos abundan en tejidos superficiales, piel, etc., y predominan en el lacón y jamones después de curados, debido a que son capaces de resistir sales, nitratos y nitritos, al mismo tiempo que a la acción de la temperatura por la presencia protectora de las grasas; *b)*, las corinebacterias, de origen desconocido, abundan en carne cruda y curada, existiendo algunas especies que resisten las variaciones de pH, la sal y temperaturas altas y bajas; *c)*, los lactobacilos que existen en carnes curadas y en conservas, resisten la sal, bajo pH y calor, y *d)*, los *Achromobacter* y *Pseudomonas* se desarrollan bien en carnes enfriadas, pero su crecimiento es afectado por el pH y la sal. Asimismo Sulzbacher (35), con-

trola mediante recuentos bacterianos las variaciones en riqueza microbiana de carnes según las diferentes temperaturas de incubación o almacenamiento ensayadas, y Kelch (16) realiza estudios parecidos, en relación con el grado de humedad atmosférica.

En relación con las alteraciones producidas y significación de la presencia de microorganismos en carnes, tenemos que, independientemente de que todo animal afectado por una enfermedad infecciosa del aparato digestivo puede infectarse muscularmente por el paso de las bacterias a través de la pared intestinal, y de que también otras enfermedades bacterianas de direfente etiología con variadas localizaciones, pueden hacer peligroso el consumo de determinadas carnes; es preciso señalar aquí la importancia que tienen otros microorganismos que, aun no siendo patógenos, provocan sin embargo marcadas alteraciones en el producto alimenticio, y por otra parte es razonable pensar que muchas de las ligeras alteraciones digestivas después de la absorción de alimentos, accidentes frecuentes, son causadas por contaminaciones banales en los productos ingeridos; los microbios de estas contaminaciones actúan mucho más por los productos de transformación que crean en el alimento—compuestos aminados, histamina, tiramina, metilaminas—que por ellos mismos. También muchas bacterias existentes en el aire o en la piel e intestinos de los animales causan fenómenos de descomposición putrefactiva, desdoblamiento de grasas o enranciamiento de las mismas, que pueden dar origen a verdaderas toxinas, y lo mismo sucede con las alteraciones provocadas por bacterias fosforescentes, (especies de *Photobacterium y Bacterium phosphorescens),* por *Bacillus prodigiosus* o por mohos. Por tanto, se comprende que al lado de la búsqueda de los gérmenes verdaderamente patógenos se debe reservar un gran espacio para la investigación de los gérmenes banales: colibacilo, *Proteus,* bacterias diversas, enterococo, etc., a su recuento aproximado y a la determinación de su actividad sobre la molécula albuminoidea. Así no es de extrañar que Hemmes (7) estudie el papel que las carnes de cerdo juegan en la propagación de una epidemia de salmonelosis paratífica B; ni que Behre (2) haga una detallada discusión de los métodos usuales empleados en la detección de envenenamientos bacterianos alimenticios, especialmente de botulismo, y que Niven (28) observe la presencia de enterococos en jamones como agentes de alteración,

aunque sin establecer, entonces, definitivamente si eran capaces de provocar síntomas de envenenamiento en el hombre.

Son interesantes los estudios de Hornsey y Mallows (10) sobre la intervención de la población bacteriana en los cambios químicos que se efectúan en el producto alimenticio; así la presencia de *Achromobacter* va asociada con la oxidación de lactatos, reducción de nitritos y disminución del potencial de óxido-reducción; sin embargo, la actuación de lactobacilos u otros gérmenes acidófilos dejan inalterable los nitratos, hay acumulación de ácido y elevación del potencial de óxido-reducción.

Referente a métodos de estudio, no hemos encontrado técnicas que personalicen de modo propio el examen bacteriológico de carnes, ya que excepción hecha de la esterilización externa por inmersión en parafina hirviente, todo lo demás son simples técnicas de Microbiología General aplicadas al examen de carnes; no obstante, señalaremos: que Pietre (29) considera interesante la determinación del pH, no por tener importancia directa, sino por su influencia en la población microbiana y por servir de índice del estado de conservación del alimento; que Majewska y Turrowski (21) establecen sus resultados sobre muestras "incubadas" y "no incubadas", análogamente a Rienel (30), que si bien hace siempre la incubación de la muestra a 37° C. fija en sus resultados las variaciones que establece en el periodo de incubación; que, según parece, para Voegeli, Bratzler y Mallmann (39), los exámenes bacteriológicos mediante recuentos bacterianos, son suficientes tan solo como mero control de la población bacteriana: y por último, que Burke, Steinkriaus y Ayres (3), desarrollan un método volumétrico y otros gravimétrico para determinar la incidencia de esporas de anaerobios putrefactivos, y Hobbs (8) esboza, para el primer Simposium Internacional de Bacteriología de los Alimentos, una marcha a seguir—microscopia directa, recuento sobre placas y cultivos directos y por enriquecimiento— sobre la que fundamenta sus experiencias. Párrafo aparte merece por su importancia en la inspección de alimentos, tanto por su rapidez como por fundarse en ensayos que no precisan de aislamiento e identificación previa de gérmenes, el "test H. I. L.", el cual será desarrollado en lugar oportuno.

Esto es cuanto hemos podido recopilar sobre Bacteriología de Carnes de Consumo en general, y en ninguno de los trabajos consultados se

hace referencia a las carnes de jabalí o venado, por lo que para este tema podemos resumir así el *estado actual del problema*:

1) No hemos encontrado antecedentes de trabajo alguno que se refieran a estudios bacteriológicos de carnes de jabalí o venado.

2) Creemos resulta interesante conocer el estado higiénico, desde un punto de vista microbiológico de carnes procedentes de estos animales—con toda seguridad sanos—que fueron abatidos en momentos que se pueden considerar como de una incrementada vitalidad, por la modalidad de caza en que son cobrados.

3) Al buscar apoyo en análogos trabajos sobre carnes procedentes de otros animales, tampoco—según nuestro criterio—hemos encontrado estudios completos en el aspecto microbiológico, ni directrices a seguir para experiencias de este tipo.

4) Dado que estas carnes a estudiar se pueden considerar exentas de gérmenes patógenos, la búsqueda ha de ir orientada a la determinación de microorganismos que no siendo capaces por sí mismos de producir enfermedades, son sin embargo causantes, por su metabolismo de sustancias tóxicas procedentes de la desintegración de proteínas cárneas.

PARTE EXPERIMENTAL

a) *Características de las muestras.*

Las muestras fueron recogidas de la canal, en bloques de aproximadamente 7 cm. de arista, tomándolas de la masa muscular de la cadera, procurando cortarlas lo más alejado posible de las heridas que el animal presentaba, a fin de evitar errores por exceso de la contaminación natural y por el aflujo de sangre que lleva consigo una mayor polución bacteriana. Sus características son las correspondientes a toda porción muscular que ha sufrido una gran congestión sanguínea.

El estudio se realizó sobre 73 gamos *(Cervus dama)* y 14 jabalíes *(Sus escrofa)*, sin distinción de edades ni sexo, aunque hubo un gran predominio de machos.

b) *Técnicas.*

Antes de exponer los métodos empleados, creemos oportuno explicar las manipulaciones que se efectuaron con las muestras para ponerlas en las debidas condiciones de efectuar las experiencias:

a) Mediante espátula metálica al rojo, se hace una esterilización de toda la superficie de la muestra, habiendo separado previamente una porción (α) para recuento global de microorganismos.

b) A continuación se practica con material estéril el tallado, en cubo geométrico de 6 cm. de arista, aproximadamente, del trozo de carne.

c) De este bloque se separan dos porciones laminares para su empleo: la mitad de una (β), para determinación del pH; y el resto (γ) de ella para preparación de frotis; la otra porción laminar (ω) es utilizada para las técnicas de recuento bacteriano.

d) El bloque principal es sumergido durante tres minutos en parafina hirviente sobre b. m., con lo que se logra una perfecta esterilización de la capa externa, que alcanza hasta una profundidad de alrededor de 3 mm., quedando englobada en el interior, con plena vitalidad, la flora microbiana propia de esta carne.

1) *Determinación de pH.*—En mortero de porcelana se hace un triturado de carne (β) facilitándose la operación mediante adición de sílice en polvo; cuando se ha logrado una masa homogénea, se añaden 10 p. de agua destilada estéril caliente, para conseguir una más perfecta maceración; se deja en reposo durante dos horas, al cabo de las cuales sobre este líquido sobrenadante se determina el pH. Los resultados obtenidos oscilaron de 5,4 a 6,2.

2) *Examen por frotis.*—Con la porción correspondiente de muestra (γ), se hicieron frotis sobre portaobjetos, exprimiendo mediante pinzas estériles la muestra. Estos frotis se tiñeron por los métodos de Gram y Wright.

3) *Recuentos globales de microorganismos.*—Con las porciones correspondientes (α), separadas para esta determinación y previamente pesadas en condiciones estériles sobre vidrio de reloj, se hace un triturado en la forma habitual antes explicada, en presencia de 10 p. de solución fisiológica estéril, para más tarde tomar porciones de 0.1 c. c. de

este líquido y sembrar en sábana sobre placas de agar-común, agar-malta, Czapeck-maltosa y medio de Brewer, incubando a 25° o a 37° C., según los casos.

4) *Recuento de flora microbiana propia, antes de incubación.*—Se practicó del mismo modo que anteriormente, pero a partir de la porción laminar correspondiente (ω) y sembrando sobre agar-suero, agar-Saboureaud y medio de Brewer.

5) *Incubación, previa esterilización externa.*—Los bloques de muestra cárnea correspondientes, se sumergen durante tres a cinco minutos en baño de parafina hirviente, antes de incubar en estufa a 37° C. du-

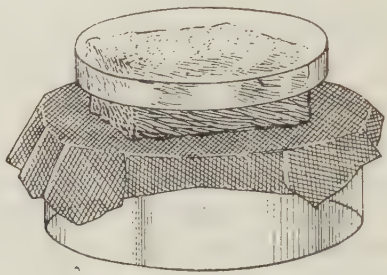


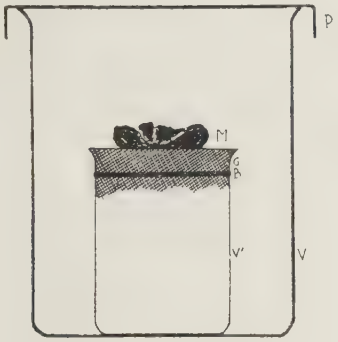
Fig. 1.

rante 24 h. según el dispositivo indicado por Nevot (Fig. 1), que nosotros hemos modificado con vistas a obtener mayor garantía contra contaminaciones accidentales externas (Fig. 2) y que no creemos necesario explicar con más detalle por considerar el esquema suficientemente claro. De esta muestra, enriquecida en gérmenes por la incubación, se parte para las sucesivas determinaciones.

6) *Examen por frotis.*— Se hace igual que en el apartado (2), pero tiñendo tan solo por el método de Gram.

7) *Cultivos sobre medios diferenciales.*—A partir de las porciones de muestra incubadas, se hicieron siembras sobre placas de E. M. B. y verde brillante; agar-sangre, Chapman y esculina; Saboureaud, y por la técnica de Reilly. Una vez aisladas las respectivas colonias, se procedió a su investigación mediante las pruebas bioquímicas que consideramos oportunas y haciendo uso del "Bergey's Manual".

8) *Test H. I. L.*—Dado que hemos considerado estas muestras, exentas de gérmenes patógenos, al hacer un estudio de la flora banal, hemos considerado opurtuno ampliar nuestro estudio a la aplicación del "test H. I. L.", propuesto por Nevot, para la investigación de gérmenes proteolíticos; estos gérmenes, según dijimos antes, son capaces de pro-



V-V' vasos de precipitados
P tapa de placa Petri
G gasa
B borde de goma
M muestra de carne

FIG. 2.

ducir intoxicaciones en el hombre, por ingestión de carnes en proceso de desintegración proteica con formación de histamina, toxinas, metilamina, etc.

Para esto, a partir del bloque de carne parafinado e incubado 24 h. a 37° C., se hace investigación de la producción de sulfhídrico e indol en los cultivos sobre medios peptonados y si hay o no liquefacción de la gelatina, empleando como inóculo una pequeña porción de esta carne incubada. Este "test H (H_2S), I (indol), L (liquefacción), da indicaciones particularmente útiles sobre la presencia de gérmenes proteolíticos en las muestras examinadas.

9) *Inoculación a animales.*—En algunos casos, también se efectuaron inoculaciones intraperitoneales en cobaya o ratón a partir del caldo peptonado que utilizamos para investigación del indol.

c) *Cuadros y resultados.*

A continuación expondremos, de modo resumido y tabulado, los resultados de las diferentes pruebas y determinaciones, que nos conducirán a la calificación definitiva de las muestras de carne analizada. Así, en relación con las más elementales determinaciones que pueden hacerse con una muestra de carne a analizar bacteriológicamente, se obtuvieron una serie de resultados que han permitido agrupar estas 87 muestras en 8 grupos, de acuerdo con el comportamiento frente al "test H. I. L.", al mismo tiempo que se indican valores de pH hallados y las cifras de recuento global de gérmenes. Por otra parte, en el cuadro correspondiente, se indican las especies encontradas, al mismo tiempo que se hacen patentes sus frecuencias de aparición y su posible localización, ya que, dado las diferencias tan grandes encontradas entre las de recuento global y las de flora bacteriana propia, es de presumir que un 75 % sea casi el producto de una contaminación accidental; por tanto, habrá especies que existirán en la capa más externa y otras —orígenes de una infección o vectores de procesos infecciosos latentes— que estarán en zonas más internas.

De todo cuanto hasta aquí se ha dicho, se deducen unas diferencias en contaminación y en frecuencias de aparición de las especies microbianas aisladas, apreciables con toda claridad en los cuadros que siguen, y en los que se puede comprobar que el *B. subtilis* y *Pr. vulgaris* son las dos especies que mas frecuentemente contaminan estas carnes, y que, especies de *Sarcina lutea, Strep. liquefaciens* y *E. coli,* son las que habitualmente se presentan como huéspedes del tejido muscular.

CUADRO I

Con relación a las muestras recibidas y examinadas.

Grupo	Procedencia	Núm. de muestras	Caracteres organolépticos	Resultados del «Test H. I. L.»	Valores de pH	Recuentos globales/g. de carne
A	Jabalí	4	Aspecto normal	H I L	5,2-5,6	2.800.000-4.200.000
	Gamo	31				
B	Jabalí	0	Aspecto normal	H I L	5,4-5,4	5.000.000-9.400.000
	Gamo	9				
C	Jabalí	2	Aspecto normal	H I L	5,1-5,4	3.200.000-6.000.000
	Gamo	9				
D	Jabalí	1	Aspecto normal	H I L	5,1-5,4	5.800.000-7.600.000
	Gamo	5				
E	Jabalí	2	Gran congestión	H I L	5,2-5,4	5.800.000-8.200.000
	Gamo	7				
F	Jabalí	1	Gran congestión y exudación	H I L	5,4-5,7	1.000.000-3.400.000
	Gamo	8				
G	Jabalí	1	Color rojo oscuro	H I L	5,3-5,8	2.600.000-7.000.000
	Gamo.....	4				
H	Jabalí	3	Color rojo oscuro, friables al tacto; congestión y exudación	H I L	5,6-6,1	3.200.000-4.000.000
	Gamo	1				

CUADRO II

Relación de muestras-especies microbianas.

ESPECIES MICROBIANAS	Frecuencia de aparición en especies				Clasificación según el área de localización en las muestras
	JABALI		GAMO		
	N.º	°/₀	N.º	°/₀	
Micrococcus pyogenes	2	15	6	8,2	Contaminación e infección.
Micrococcus ureae	1	7,5	—	—	Contaminación.
Micrococcus flavus	1	7,5	7	9,1	Idem.
Sarcina lutea	8	57,1	52	71,2	Contaminación e infección.
Streptococcus zymogenes	3	21,4	12	16,7	Idem.
Strep. Liquefaciens	5	35,7	9	12,3	Idem.
Corynebacterium (saprof.)	—	—	5	6,8	Contaminación.
Cory. liquefaciens	1	7,5	—	—	Infección.
Cory. renale	—	—	2	2,7	Idem.
Pseudomonas denitrificans	—	—	2	2,7	Contaminación.
Pseudomonas fluorescens	1	7,5	—	—	Idem.
Proteus vulgaris	4	28	11	15	Idem.
Paracolobactrum	—	—	14	19,1	Infección.
Escherichia coli	3	21,4	19	27,3	Contaminación e infección.
Salmonellas saprofitas	4	28,5	—	—	Idem íd.
Lactobacillus brevis	2	13	—	—	Contaminación.
Bacillus pseudoanthracis	—	—	7	9,5	Idem.
Bacillus cereus	—	—	4	5,4	Idem.
Bacillus megatherium	—	—	10	13,6	Idem.
Bacillus subtilis	5	35,7	48	65,7	Idem.
Clostridium bifermentans	1	7,5	3	4,1	Idem.
Candida parakrusei	—	—	1	1,3	Idem.
Candida Guillermondii	—	—	1	1,3	Idem.
Aspergillus sp.	—	—	1	1,3	Idem.

CUADRO III

Con relación a algunos valores límites hallados.

Especies animales	Recuentos flora bacteriana propia/gr.	Recuentos anaerobios globales/gr.	Porcentaje microorganismos no bacterianos	Porcentaje de especies patógenas para cobaya
Jabalí	15.000 a 800.000	180.000 a 1.200.000	0	20 °/₀
Gamo	64.000 a 1.300 000	110.000 a 900.000	4,1 °/₀	5 °/₀

CUADRO IV

Sentido de la frecuencia de las especies cárnicas propiamente dichas.

%	57,1	35,7	28,5	21,4	15	7,5
Jabalí	*Sarcina lutea.*	*Streptococcus liquefaciens.*	*Salmonellas saprofitas.*	*Strep. zymogenes y Escherichia coli.*	*Micrococcus pyogenes.*	*Corynebacterium liquefaciens.*

Gamo	*Sarcina lutea.*	*Escherichia coli.*	*Streptococcus zymogenes.*	*Strep. liquefaciens.*	*Micrococcus pyogenes.*
%	52	27,3	16,7	12,3	8,2

CUADRO V

Sentido de la frecuencia de las especies de contaminación accidental.

%	35,7	28	15	7,5
Jabalí	*Bacillus subtilis.*	*Proteus vulgaris.*	*Lactobacillus brevis.*	*Micrococcus ureae, Mic. flavus Pseudom. fluorescens y Cl. bifermentans*

Gamo	*Bacillus subtilis.*	*Proteus vulgaris.*	*B. megatherium.*	*M. flavus y B. pseudoanthracis.*	*Coryn. saprof.*	*B. Cereus*	*Pseud. denitrif.*	*Cl. bifermentans. Candida parakrusei, G. Guillerm. Asp.*
%	65,7	15,03	13,6	9,5	6,8	5,4	2,7	1,3

Por último, se hace una clasificación de las 84 muestras examinadas, según se puede ver en el cuadro VI, desde un punto de vista de ordenación higiénica en correspondencia a los resultados del "test H. I. L.", el cual, pese a no estar reconocido en las leyes sanitarias de nuestro país, es considerado por nosotros de gran interés para la inspección de cualquier clase de carnes. Bien es verdad que muchas carnes consideradas inadmisibles en presencia de resultados de esta prueba, pueden ser consumidas sin que necesariamente se hayan de producir perturbaciones digestivas, ya que se trata de gérmenes no patógenos; grupo, el patógeno, que se ha dejado fuera de este estudio, porque su presencia es motivo suficiente de decomiso de las carnes que los contengan. Ahora bien, señalamos una vez más, que, en nuestro criterio, la inspección sanitaria de carnes debe conducirse a un grado de máxima seguridad y ampliarse en el sentido de la investigación de gérmenes capaces de producir intoxicaciones derivadas de las sustancias que se originan en su metabolismo, ya que el porcentaje de carnes calificadas como inadmisibles por estas causas, es lo suficientemente elevado para obligar a tenerle en cuenta.

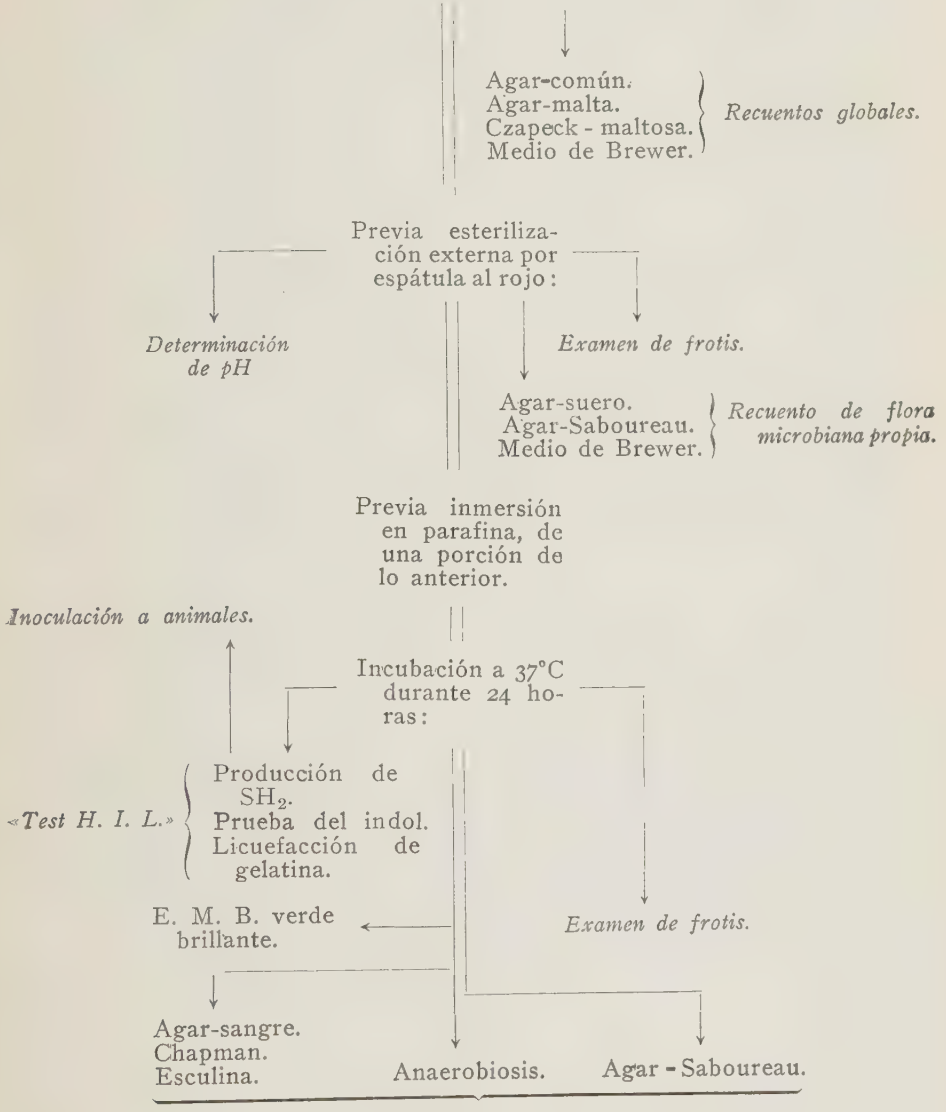
CUADRO VI

Grupo	Resultados del Test	FRECUENCIAS EN %		CALIFICACION
		JABALÍ	GAMO	
A	H I L	28,4	42,4	Buena.
B	H I L	0	12,3	Buena.
C	H I L	14,2	12,3	Admisible.
D	H I L	7,1	6,8	Mala.
E	H I L	14,2	9,5	Admisible.
F	H I L	7,1	10,9	Mala.
G	H I L	7,1	5,4	Inadmisible.
H	H I L	2,1	1,3	Inadmisible.

Con lo hasta aquí expuesto, consideramos haber realizado un estudio suficientemente completo sobre la inspección microbiológica de carnes de jabalí y venado, al mismo tiempo que presentamos a continuación la marcha que consideramos más oportuna para el control bacteriológico de carnes, que, según la experiencia adquirida en este trabajo, podemos afirmar suministra datos eficientes en cuarenta y ocho horas, tiempo bastante rápido, si se tiene en cuenta la obligada demora de todas las pruebas bacteriológicas.

ESQUEMA QUE SE PROPONE PARA EL EXAMEN BACTERIOLOGICO DE CARNES

MUESTRA DE CARNE REMITIDA PARA SU EXAMEN:



Cultivos diferenciales para aislamiento.

d) *Consideraciones sobre los resultados.*

En relación con los caracteres organolépticos hemos podido observar que en color y aspecto eran normales, excepto en algunas muestras —las más positivas ante las tres pruebas del test en estudio— que el color rojo normal estaba intensificado y con alto grado de congestión sanguínea.

Los valores encontrados para pH son mas bajos que los citados en la bibliografía, pudiéndose explicar esto por el predominio de metabolitos ácidos producidos en el metabolismo muscular consecuente al trabajo ejecutado por el animal en los momentos anteriores a su muerte por caza en campo abierto.

Poco pueden significar las cifras de recuentos globales —tanto de aerobios como de anaerobios— en cuanto al estudio bacteriano en sí, pues estas cifras pueden aumentar o disminuir en sentido geométrico, dependiendo de las condiciones de transporte y despiece de las reses; teniendo en cuenta además, que estos gérmenes son superpuestos a la superficie de la carne, de la cual lógicamente desaparecerán en el cocinado de la misma. Por el contrario, han de tenerse en cuenta los datos encontrados referentes al número de colonias/gr. para la flora bacteriana propia, porque estos sí tienen significación en el grado de polución.

Las especies más frecuentemente halladas son: *Sarcina lutea, Strep. liquefaciens* y *E. coli,* como gérmenes incluídos en el espesor muscular, y *B. subtilis* y *Pr. vulgaris,* como contaminantes superificiales y de aparición accidental.

En la calificación higiénica no hemos hallado —tal como esperábamos, dada la vitalidad de las piezas cobradas—, germen patógeno alguno. Hemos hecho ocho grupos, con arreglo a todas las posibilidades de combinación de las tres pruebas del "test H. I. L." y hemos asignado las calificaciones de buena, admisible, mala e inadmisible, de acuerdo con las positividades de las diferentes pruebas del test.

RESUMEN

1.—Se recogen antecedentes bibliográficos referentes a Bacteriología de Carnes en general, ya que no se han encontrado citas bibliográficas que se refieran a estudios similares en jabalí y gamo, con lo que creemos actualizar el tema que nos ocupa.

2.—Se han realizado exámenes bacteriológicos correspondientes a 87 muestras de carnes procedentes de caza mayor (14 de jabalí y 73 de gamo).

3.—Se hace un control analítico de estas muestras mediante el "test H. I. L." —producción de H_2S(H), producción de indol (I) y liquefacción de la gelatina (L)— para la detección de gérmenes proteolíticos, distribuyéndolos en ocho grupos según los resultados.

4.—A la vista de los resultados obtenidos, se propone el uso del "test H. I. L.", como práctica habitual en el control bacteriológico de las carnes.

5.—Se propone una marcha de trabajo para estudios de este tipo, con la que hemos obtenido mejores y más rápidos resultados.

SUMMARY

In this work, the autor has summarized the bibliographic antecedents referring to Meat Basteriology in general; he did not find reports about similar studies on wild boar and game.

There have been made bacteriological examinations on 87 samples of meat proceeding from big game (14 of wil board and 73 of game) and at the same time the "Test H. I. L." for the bacteriological control of these meats has been applied, the good results of which are very useful for these purposes.

At last a method to work is presented which is recommended for studies of this kind because with it, better and quicker results are obtained.

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ESTUDIO SOBRE EL METABOLISMO DE LOS ACIDOS NUCLEICOS DE LAS BACTERIAS

II. Metabolismo de los ácidos nucleicos del *Escherichia coli* en medio sintético.

POR

RAMONA VAAMONDE y BENITO REGUEIRO

Conocida es la importancia de los ácidos nucleicos en la constitución y vida de todas las células de los organismos superiores e inferiores. Si fundamentales son en las células vegetales y animales, más todavía lo son en las células bacterianas, en las que proporcionalmente entran en mayor cantidad en su constitución.

En un trabajo anterior (I), hemos realizado el estudio del crecimiento de *Escherichia coli* y de la *Sarcina lutea* en medio de caldo común a diferentes temperaturas y condiciones de aireación. Al mismo tiempo hemos estudiado el aislamiento y la determinación de los ácidos nucleicos (RNA y DNA) en diferentes fases de crecimiento de los dos gérmenes mencionados (lag, logarítmica, estacionaria y declive), estableciendo las curvas de biosintesis de dichos ácidos nucleicos, que, en cierto modo, coinciden con las de autores anteriores (2, 3).

Sabido es que los microorganismos sintetizan sus componentes, a partir de los elementos nutritivos del medio. Cuando éste es del tipo de caldo común, en el cual su composición es compleja y variable, es difícil el estudiar la influencia que un determinado compuesto puede ejercer en la formación de una determinada sustancia por el microorganismo; en nuestro caso de los ácidos nucléicos. Es por esta razón que recurrimos a un medio de conocida composición, en el cual pueda desarrollarse el germen, para ver cómo influyen los componentes del mismo en el crecimiento y en la formación de los ácidos nucleicos. Este es el objetivo del presente trabajo.

TECNICAS

En este trabajo se utiliza solamente el *Escherichia coli,* sembrado a partir de tubos inclinados de agar - caldo, durante veinticuatro horas, a 37° C. El medio sintético que utilizamos para este germen posee la composición siguiente: 0,20 % de glucosa; 0,80 % de sulfato amónico; 0,20 % de fosfato monopotásico; 0,30 % de fosfato dipotásico; 0,07 % de sulfato de magnesio, y 0,03 % de sulftao de hierro. Se pone a pH, 7,2.

Las determinaciones de crecimiento se realizan por los métodos expuestos en el trabajo anterior, determinando turbidez en fotocolorimetro. La preparación de gérmenes desecados, también por los métodos expuestos, deshidratando con acetona y éter.

El aislamiento de los ácidos nucleicos se realiza por el método de Schmidt y Thannhauser (4), modificado por Sherra y Jones (5). La determinación se hace por reacciones de: fósforo total, por el método de Fiske y Subbarow (6); ribosa por el orcinol, por el método de Mejbaum (7), y deoxiribosa por la difenilamina, por el método de Dische y Schwarz (8).

RESULTADOS

1.° Lo primero que tratamos de ver es la curva normal de crecimiento del *Escherichia coli* en el medio sintético completo, en agitación a 28°. Los resultados obtenidos, expresados gráficamente en turbidez (extinción), número de gérmenes y peso seco, se presentan en la figura 1.

Observando la composición del medio sintético, se ve que los fosfatos tienen la función de controlar el pH por su efecto tampón, luego no deben de influir fundamentalmente en la cantidad y número de gérmenes de una manera directa. Son los otros componentes: azúcar, nitrógeno, magnesio y hierro, los que deben tener dicho efecto directo y fundamental. Por esta razón tratamos de cuál es la mínima cantidad de cada uno de estos elementos que puede sostener el crecimiento. En el cuadro siguiente, se expresan las cantidades de cada elemento añadidas al medio, inoculado con cultivo de veinticuatro horas de *Escherichia coli* y puestos en agitación a 28°:

Glucosa — Por 100	Nitrógeno — Por 100	Magnesio — Por 100	Hierro — Por 100
0,00	0,00	0,00	0,000
0,10	0,20	0,01	0,001
0,20	0,40	0,03	0,002
0,30	0,60	0,05	0,003
0,40	0,80	0,07	0,004
	1,00	0,09	0,005
	1,20	0.11	0,006

En estos casos, los resultados se dan en turbideces (extinción) y se expresan en las figuras 2-5.

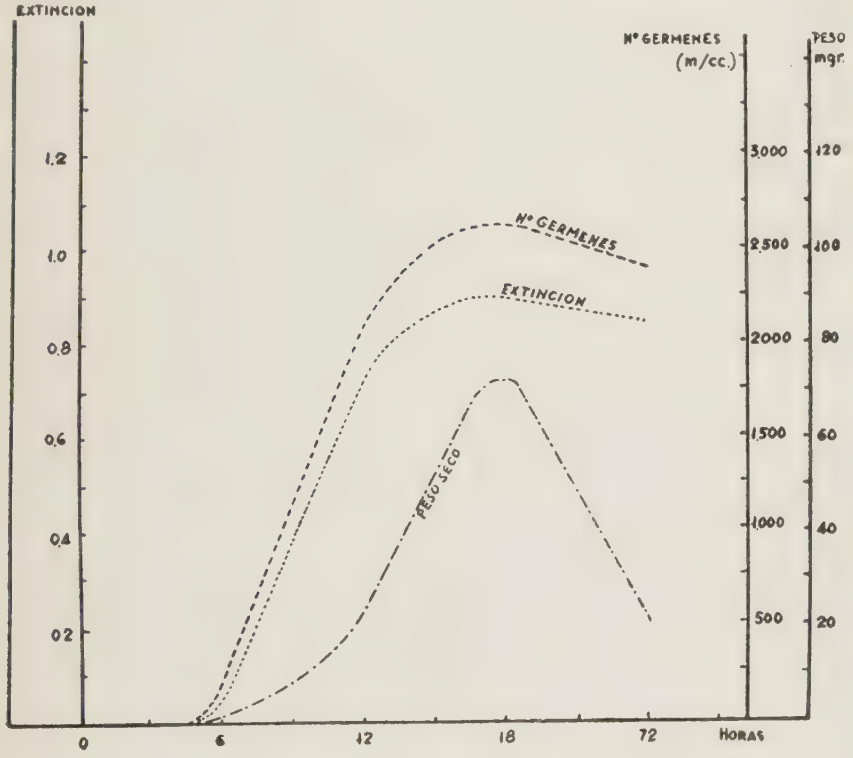

FIG. 1.

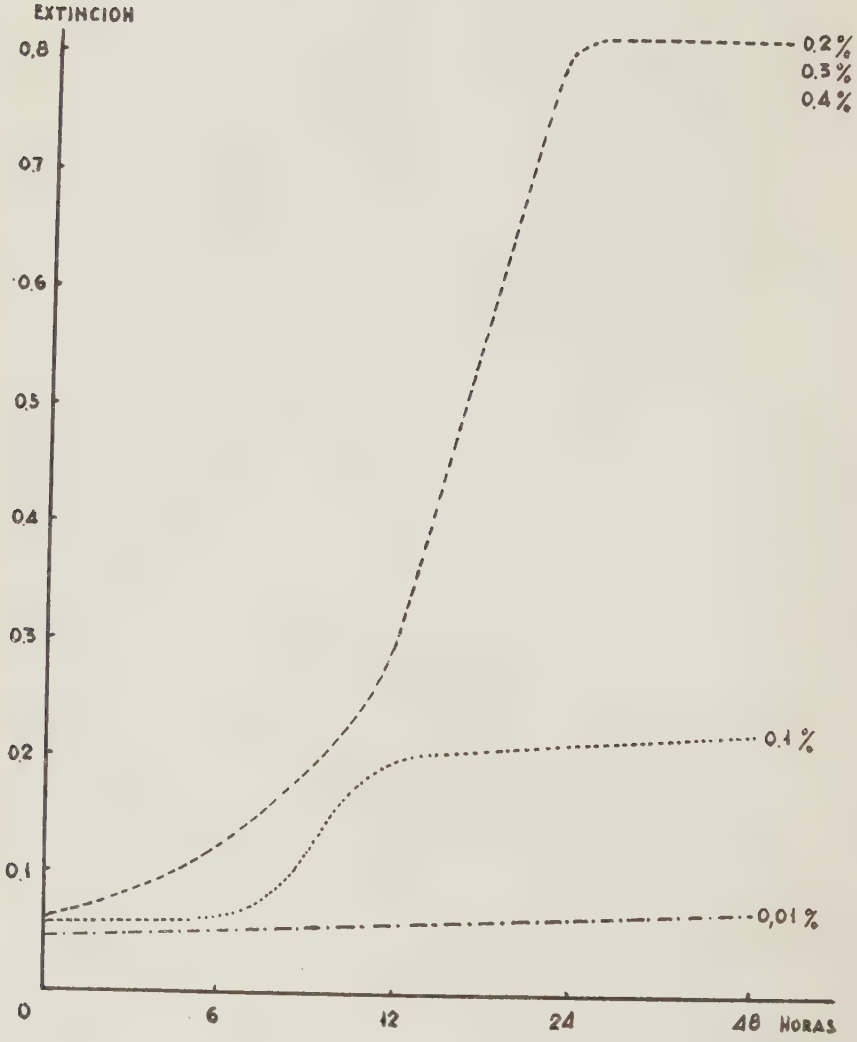

FIG. 2.

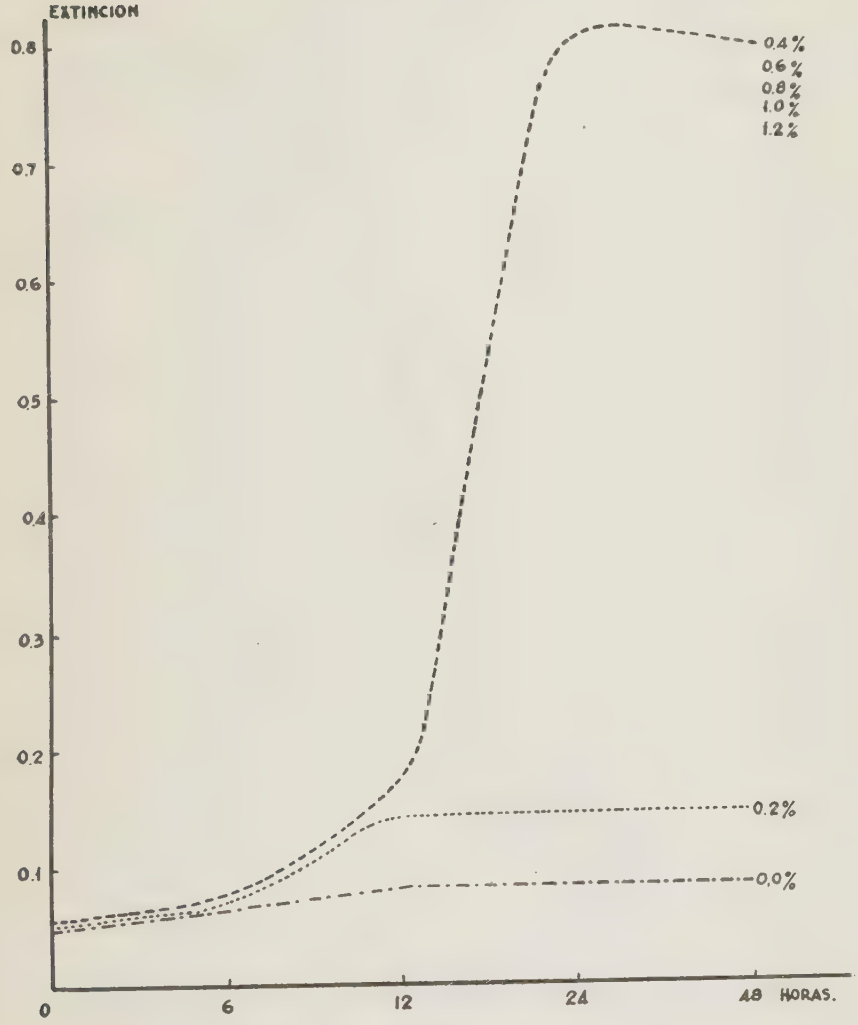
EXTINCION
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
12
24
48 HORAS.
0.4 %
0.6 %
0.8 %
1.0 %
1.2 %
0.2%
0.0%

FIG. 3.

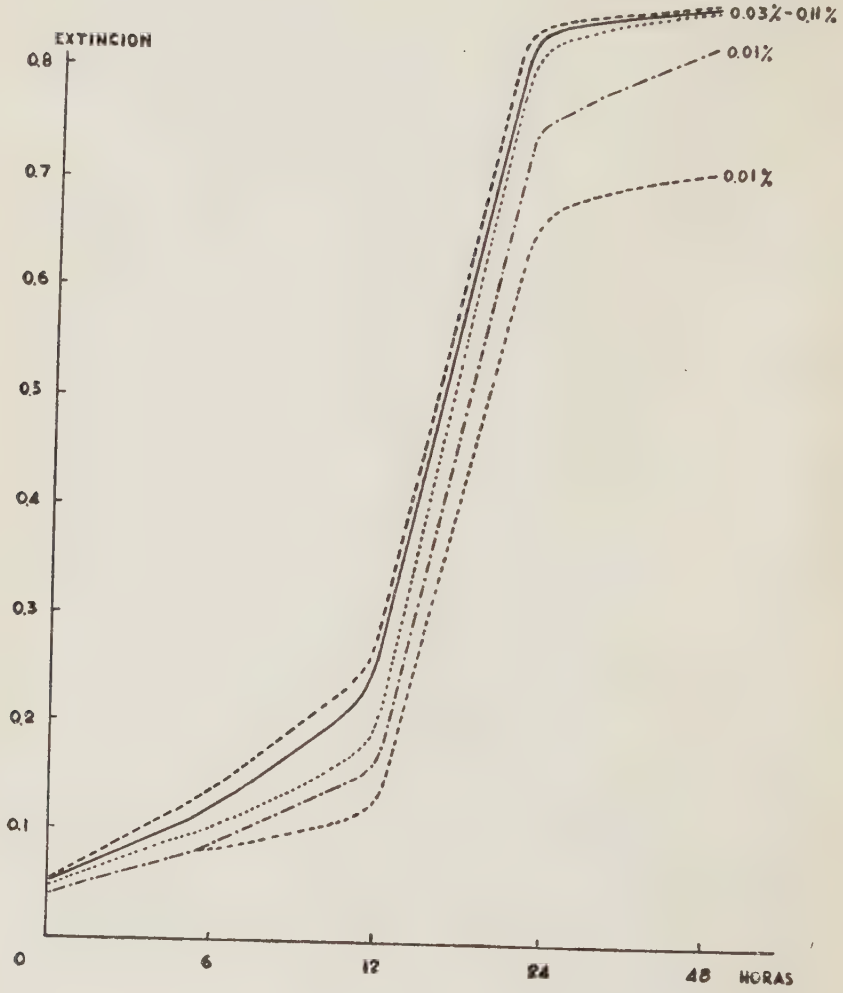
EXTINCION
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
12
24
48
HORAS
0.03%-0.11%
0.01%
0.01%

FIG. 4.

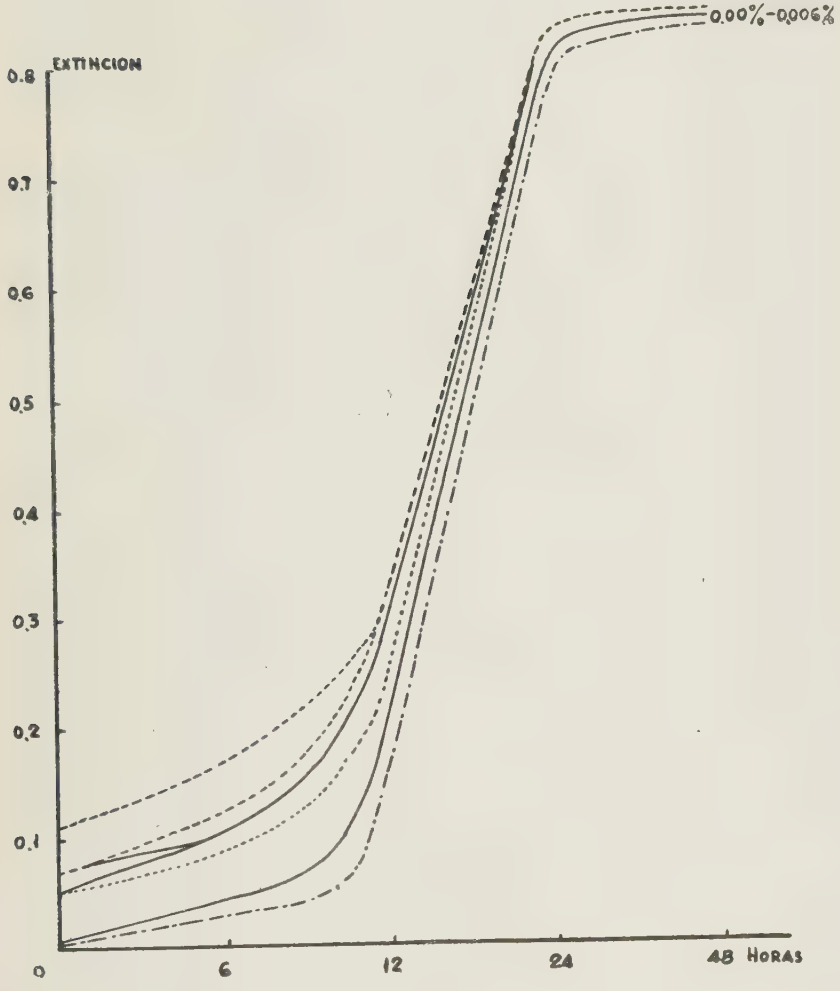

FIG. 5.

De la observación de estas curvas se deduce que, para que se desarrolle un crecimiento parecido al normal, se necesita, por lo menos, un 0,2 % de glucosa, la cual es la cantidad que se añade al medio normal. De sulfato amónico se necesita un 0,4 %, lo cual representa la mitad de lo que se añade al medio normal completo. En lo relativo a las cantidades de hierro y magnesio, si pueden tener alguna influencia en la primera fase de crecimiento, no se observan variaciones a partir de las doce horas de crecimiento en ninguno de los casos.

2.° A continuación se trata de aislar y determinar los ácidos nucleicos del *Escherichia coli* en medio sintético completo, en diferentes fases de crecimiento en agitación a 28°. Las determinaciones se hacen en el líquido alcalinõ, del cual se separan después las dos fracciones de ácidos (RNA y DNA). Los resultados del líquido alcalino son los siguientes:

Horas	Fósforo — Por 100	Nucleicos Por 100	RNA — Por 100	DNA — Por 100
6	3,2	37,5	48,0	23,2
12	2,9	34,1	20,8	10,0
18	2,3	27,0	16,3	7,9
72	2,1	24,7	16,4	7,9

Una vez separadas las dos fracciones nucleicas por los métodos conocidos, encontramos que los resultados de cada fracción son los siguientes:

Fracción RNA:

Horas	Fósforo — Por 100	RNA — Por 100	DNA — Por 100
6	1,50	10,80	—
12	1,40	2,50	—
18	1,25	2,30	—
72	1,20	1,80	—

Fracción D N A :

Horas	Fósforo — Por 100	D N A — Por 100	R N A — Por 100
6	0,20	—	—
12	0,24	2,16	—
18	0,30	3,10	0,61
72	0,30	3,00	—

De todos los anteriores resultados interesa, sobre todo, expresar gráficamente el contenido de ácidos nucleicos en el líquido alcalino, pues en las fracciones correspondientes a cada ácido, pueden existir impurezas, como se observa en el caso de la fracción DNA. (Fig. 6.)

De la observación de estas curvas se deriva que la máxima produc-

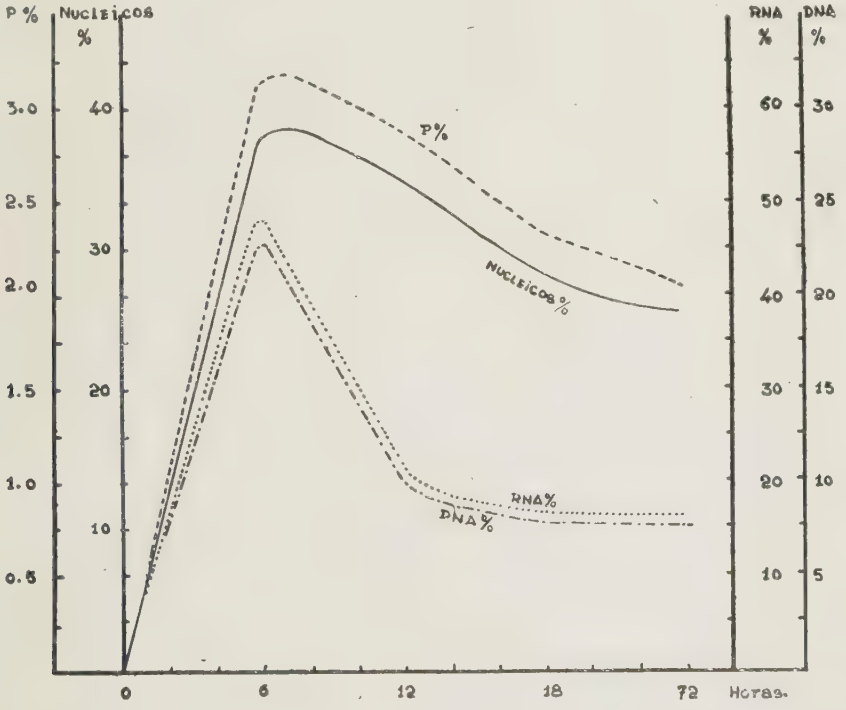

Fig. 6.

ción de ácidos nucléicos totales ocurre a las seis horas, aproximadamente, de crecimiento; es decir, al final de la fase "lag" y comienzo de la "logarítmica", disminuyendo luego durante esta fase y manteniéndose luego a un mismo nivel hasta el final. La producción de los dos áci-

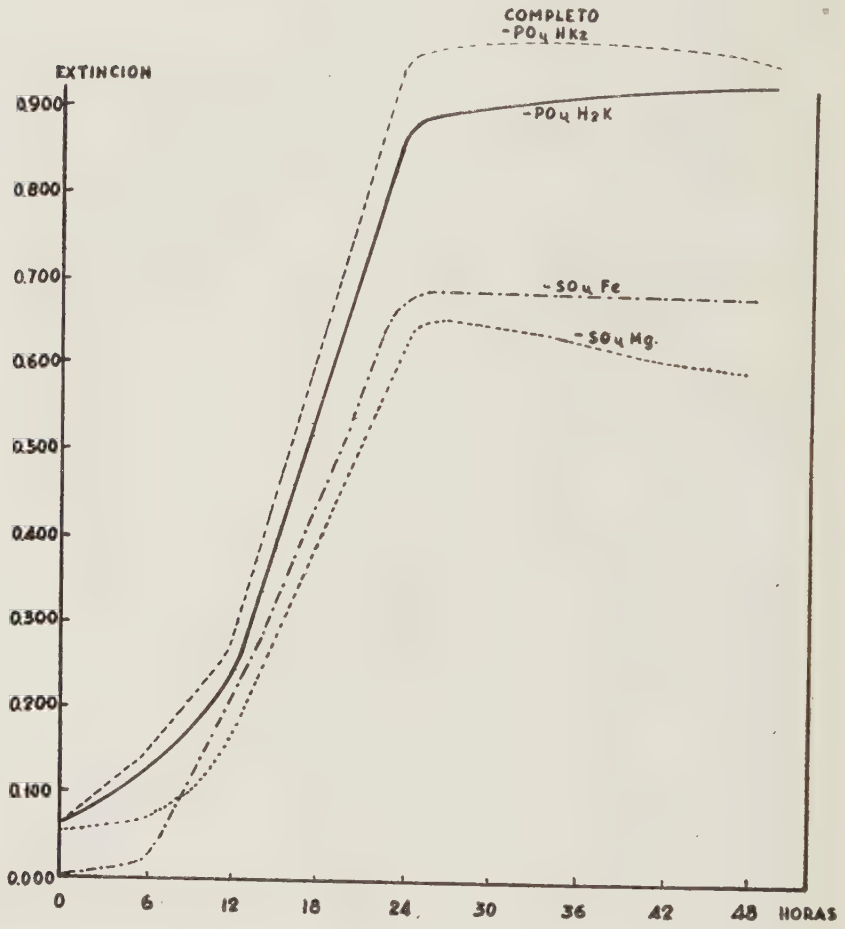

Fig. 7.

dos: RNA y DNA, se observa es paralela. Las curvas son de la misma forma que las producidas en el crecimiento del *Escherichia coli* en caldo común.

3.° Cuando estudiamos la influencia de la composición del medio sintético sobre el crecimiento del *Escherichia coli,* observamos que, tanto la glucosa como el sulfato amónico, eran componentes imprescindibles y sin los cuales no podía desarrollarse el microorganismo, Los otros cuatro componentes del medio, complementan la acción de los anteriores, pero sin ser imprescindibles. Interesa saber si tales compuestos influyen en la formación y contenido total de los ácidos nucleicos.

Lo primero que hacemos es construir las curvas de crecimiento del *Escherichia coli* en medios sintéticos: completo y sin alguno de sus componentes no esenciales (Fig. 7.)

Tomamos los cultivos anteriores de cuarenta y ocho horas, y a partir de una determinada cantidad de cada uno ,aislamos los ácidos nucleicos en el líquido alcalino y los determinamos. Los resultados de esta experiencia son los siguientes:

Medio	Peso seco — Miligramos	Fósforo — Por 100	Nucleicos — Por 100	R N A — Por 100	D N A — Por 100
Completo	65,6	1,98	23,20	30,50	4,30
$-PO_4H_2K$	67,9	1,80	21,10	21,40	3,57
$-PO_4HK_2$	55,0	1,82	21,40	20,60	3,90
$-SO_4Mg$	10,2	1,71	20,10	15,70	2,40
$-SO_4Fe$	40,1	1,72	20,20	17,30	3,00

Gráficamente se pueden expresar estos resultados según la figura 8, donde los números representan el orden de los medios que se indica.

Observando las cantidades totales anteriores, se ve que el magnesio tiene gran influencia en el peso seco de las células producidas y que el hierro la tiene mucho menor, no influyendo sensiblemente los fosfatos.

En cuanto a la producción de ácidos nucléicos, se observa que la ausencia de hierro y magnesio reduce la producción normal en un 15 %, aproximadamente. Los fosfatos casi no influyen en la producción de ácidos nucleicos.

La falta de magnesio o de hierro en el medio sintético reduce en un 50 % la producción de RNA ; en menor proporción lo reducen los medios en que faltan fosfatos.

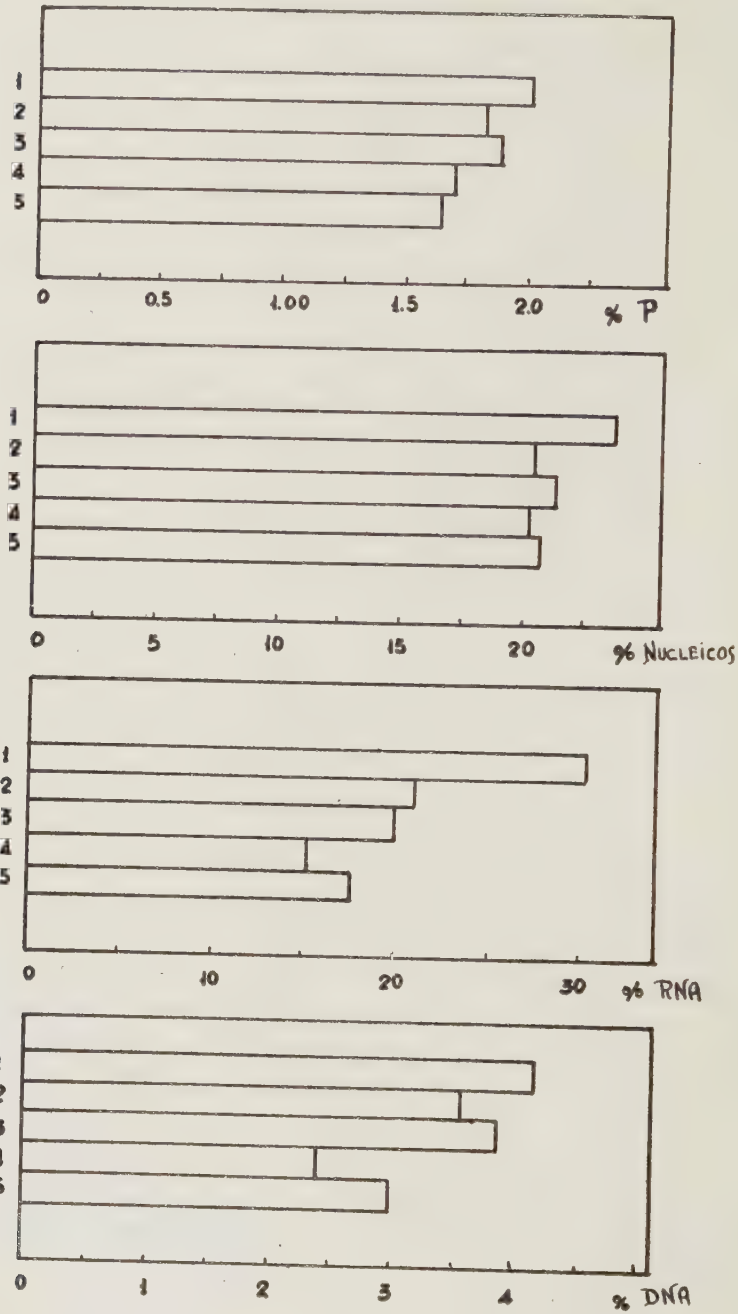
1
2
3
4
5
0
0.5
1.00
1.5
2.0
% P
0
5
10
15
20
% NUCLEICOS
0
10
20
30
% RNA
0
1
2
3
4
% DNA

FIG. 8.

En cuanto al DNA, solamente influye en su producción la ausencia de magnesio, sobre todo, que lo reduce en un 45 %. Los otros componentes influyen menos.

RESUMEN

En un primer trabajo habíamos estudiado la formación de los dos ácidos nucleicos (RNA y DNA), en diferentes fases de crecimiento del *Escherichia coli* en caldo común. Como lógicamente se comprende, la síntesis de los componentes celulares se realiza a partir de los elementos presentes en el medio de cultivo. Se hace necesario el estudiar la formación de los ácidos nucleicos del *Escherichia coli* en un medio sintético, para ver la influencia que los componentes del mismo tienen en dicha formación.

Se construyen las curvas de crecimiento del *Escherichia coli* en medio sintético completo o en ausencia de alguno de sus componentes, observándose que son esenciales: glucosa y sulfato amónico, y no esenciales: fosfato monopotásico, fosfato dipotásico, magnesio y hierro.

Se determinan los ácidos nucleicos (RNA y DNA) y se observa que la curva de su formación es parecida a cuando se realiza en caldo común, con una máxima producción al final de la fase "lag" y comienzo de la "logarítmica", disminuyendo durante esta fase y manteniéndose luego en un mismo nivel.

Cuando observamos la influencia que pueden tener los componentes del medio sintético del *Escherichia coli* en la producción y formación de los ácidos nucleicos, vemos que la ausencia de fosfatos casi no posee influencia sobre dicha formación; en cambio, la ausencia de hierro, y sobre todo de magnesio, sí ejercen gran influencia en cuanto a la formación de RNA, y la ausencia de magnesio influye en la formación de DNA.

De aquí parece derivarse qué el magnesio, en el caso del RNA y DNA, y el hierro, en el caso del RNA, toman parte en su biosíntesis, seguramente a través de complejos de enzimas.

SUMMARY

It is follows the growth of *E. coli* in basal synthetic medium, as well as without some of the components. It is found that glucose and ammonium sulphate there are esential; whereas monopotassium phosphate, bipotassium phosphate, magnesium and iron there are not essential.

The formation of RNA and DNA is similar in nutrient broth. There are a maxima production between the end of the lag and the beginning of logaritmic phase, further in this phase fall down to remain constant.

The dependence of the production of nucleic acids by *E. coli* on the synthetic media was studied. It is found that the formation of nucleic acids is almost independent of the presence of phosphate, while the absence of iron and specially magnesium have a great influence on the RNA formation. In the other hand, magnesium affect the building of DNA.

It seems that magnesium is necessary for the biosintesis of RNA and DNA probably through the enzymatic systems. It seems that iron play the same role in the DNA formation.

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ACTAS DE LA SOCIEDAD

Acta de la Sesión celebrada en Madrid por la Sociedad de Microbiólogos Españoles, el día 22 de noviembre de 1957. Homenaje a la memoria del Profesor Arnaldo Socias Amorós.

Bajo la Presidencia de D. Gerardo Clavero del Campo, y actuando como Secretario, D. Lorenzo Vilas, se abre la Sesión a las diecinueve horas y cuarenta minutos en el Aula del edificio central del C. S. I. C., Serrano, 117.

El Sr. Presidente da cuenta de las adhesiones recibidas. D. Jaime del Campo destaca las cualidades humanas del Prof. Socias, gran admirador del Dr. Ferrán. Su labor directiva, tanto en la Sociedad, como en el Instituto "Jaime Ferrán", de Microbiología, del C. S. I. C., y su vocación por las tareas investigadoras, se hacen resaltar por D. Lorenzo Vilas. D. Maximino San Miguel de la Cámara traza una semblanza del Prof. Socias como Catedrático de la Facultad de Ciencias. Finalmente, el Sr. Presidente expone la importancia de los trabajos epidemiológicos cumplidos por quien fuè figura destacada de la Sanidad nacional.

Se levanta la Sesión a las veinte horas y treinta y cinco minutos.

Depósito legal M. 702. 1958

Artes Gráficas REYES. - Jerónima Llorente, 15. - MADRID

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